International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Communications and Publications Division (CPD) of the IFCC

December 2024
Volume 35
Number 4
ISSN 1650-3414

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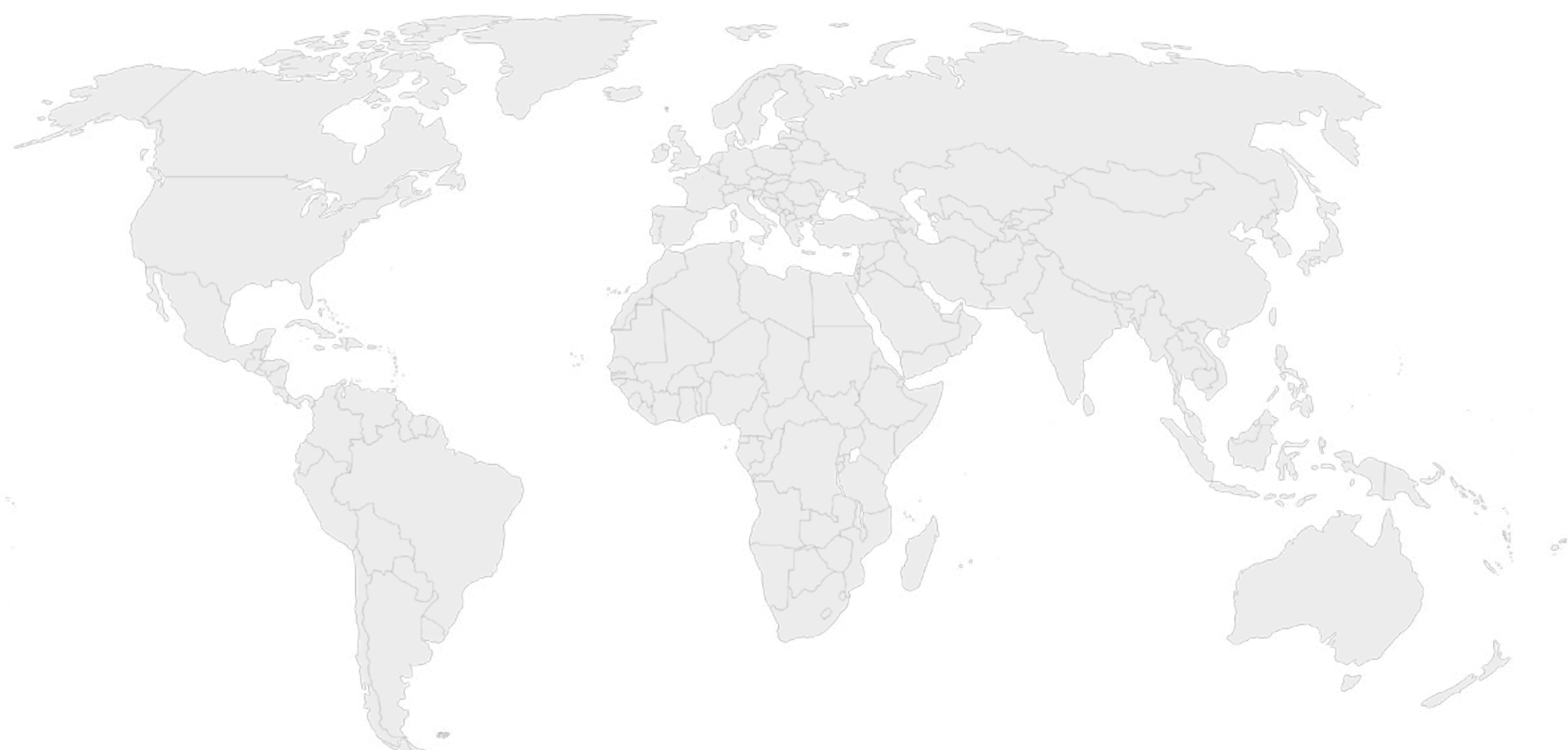

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Letter to the Editor

Light After Armageddon: Enhancing Cardiovascular Care through Emerging Technologies and Patient-Centric Approaches

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Keywords

Cardiovascular diseases, prevention, emerging technologies, artificial intelligence, biomarkers

Abstract

To the Editor,

In the wake of a global healthcare crisis that shook the very foundations of our medical systems, a metaphorical ‘Armageddon’, the world stands at a critical juncture. This juncture, while marked by challenges, also opens a doorway to unprecedented opportunities, particularly in the realm of cardiovascular care. The Covid pandemic was a first trigger of reflection with an article entitled “COVID-19: Armageddon before light” [1]. The “Light After Armageddon” initiative emerges as a beacon of hope, embodying a collective endeavor to navigate through the storm and lead cardiovascular healthcare into a new era marked by innovation, resilience, and patient-centred care. The term ‘Armageddon’, often evoking images of final battles or cataclysmic destruction, in this context, symbolizes a turning point—a crisis that demands a transformative approach in healthcare. This crisis has underscored the fragility of our health systems, revealing vulnerabilities in dealing with cardiovascular diseases (CVDs), the leading cause of mortality globally [2]. However, it has also highlighted the indomitable spirit of the medical community and the infinite potential of emerging technologies to revolutionize healthcare [3,4]. CVD, encompassing a range of conditions affecting the heart and cardiovascular system, have long posed significant challenges to healthcare providers and patients alike. Traditional approaches, while effective to a degree, often fall short in addressing the complexities of CVDs, especially in early detection and personalized treatment. The advent of the ‘Armageddon’ has acted as a catalyst, accelerating the integration of innovative solutions into cardiovascular care. These technologies,

once the realm of science fiction, are now at the forefront of a healthcare revolution, offering new pathways to diagnose, treat, and manage CVDs more efficiently and effectively than ever before [5-7]. Furthermore, emerging technologies are essential to address the increasing number of high-risk individuals and to tackle new risk factors that continue to emerge in the field of cardiovascular health [8]. These advancements are also crucial in managing and mitigating the risks posed by novel environmental and genetic factors influencing cardiovascular diseases [9].

The “Light After Armageddon” initiative stands as a sharing a vision to the power of innovation and collaboration in the face of adversity. By harnessing the capabilities of emerging technologies, the project seeks to pave the way for a future where CVD care is not only more accessible and equitable but also more attuned to the needs and expectations of patients. Through the feedback of the participants, we explored the transformative impact of these technologies on cardiovascular healthcare. We examined the successes and challenges, the stories of hope and the visions for the future, shared by leading experts and patient advocates. Through their insights and contributions, we pieced together a mosaic of possibilities, illustrating how the post-’Armageddon’ era could herald a new dawn for cardiovascular care—one where every heartbeat matters, and no patient is left behind.

First, we began by identifying a diverse group of expert stakeholders, including cardiologists, healthcare policymakers, technology developers, and patient advocates. Through a series of contributions, we gathered insights and expectations concerning new technological solutions. This initiative framework ensured a holistic understanding of emerging technologies’ role in enhancing patient outcomes, optimizing clinical workflows, and fostering a patient-centric approach in the new era of cardiovascular healthcare. The results of the collected insights unveiled compelling evidence of the transformative power of emerging technologies in cardiovascular care. The diverse contributions received painted a vivid picture of a healthcare landscape on the cusp of revolution, driven by the integration of digital innovation and patient-centric approaches.

From the contributions, a consensus emerged on the pivotal role of artificial intelligence (AI) in revolutionizing diagnostics and treatment strategies. AI’s ability to analyse large datasets has led to more accurate and rapid diagnosis, tailoring treatment plans to individual patient profiles, and significantly improving outcomes. It has also allowed for portable, AI-enhanced devices that can be used by allied healthcare professionals, such as in echocardiogram. In this fashion, it promises to dramatically expand the reach of diagnostic services across our communities and remove the unacceptable burden of adverse outcomes experienced by symptomatic patients on waiting lists for diagnosis and therefore the onset of therapies. New laboratory digital platform that integrates clinical decision support tools with hospital IT systems, highlight how such technologies enhance the precision and efficiency of cardiac care.

AI is also at the forefront of personalized care. Shifting from cure to care means that individuals with enhanced cardiovascular risks could undergo proactive treatment, even in asymptomatic cases. Such an approach promises to catch potential diseases before they manifest into symptomatic and possibly severe conditions, thereby improving outcomes and reducing long-term healthcare costs. In addition to traditional risk factors, emerging biomarkers and novel genetic markers are showing promise in identifying at-risk individuals more accurately. Incorporating these markers into routine screenings could significantly enhance early detection efforts, ultimately reducing the waiting lists for cardiovascular care by identifying and managing high-risk individuals before they develop severe symptoms. The integration of advanced biomarkers and AI algorithms in routine diagnostics can streamline patient prioritization. By accurately identifying those at highest risk, healthcare providers can start earlier the right treatment and optimize resource allocation, ensuring that high-risk patients receive timely interventions while reducing unnecessary tests and procedures for those at lower risk

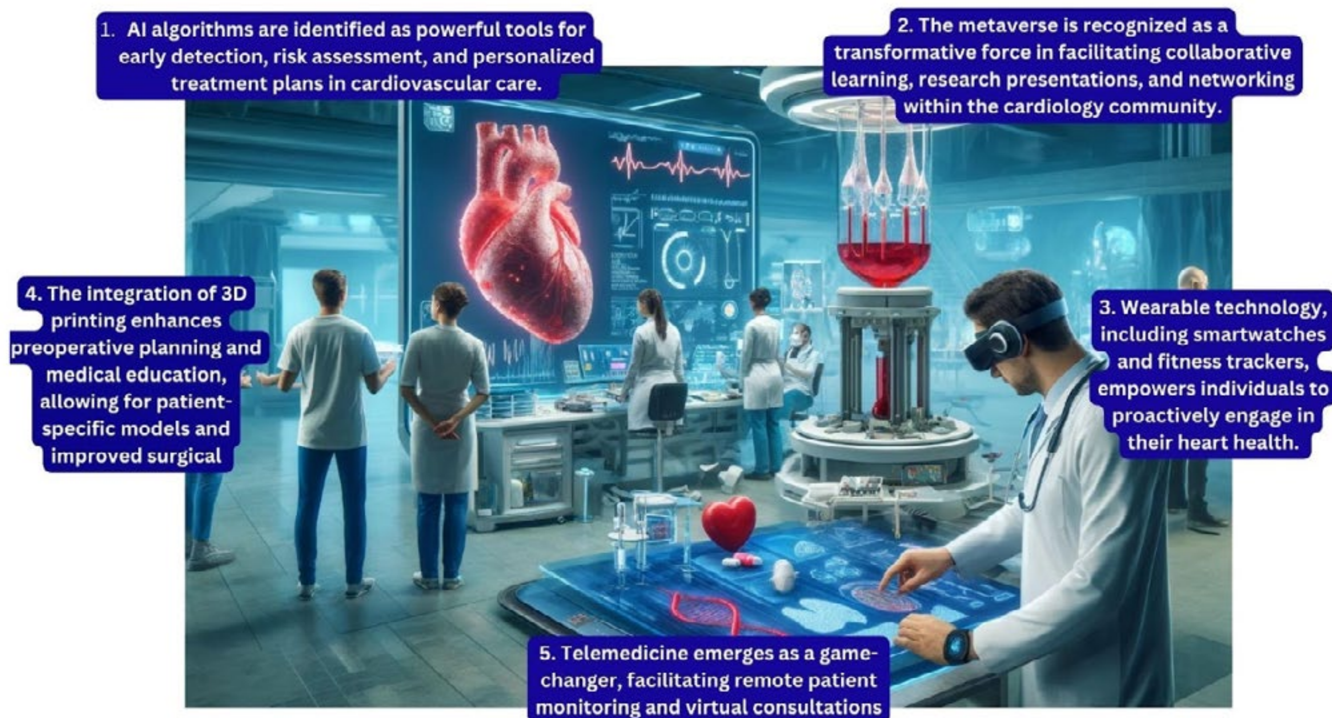
Telemedicine emerged as another crucial technological advance, breaking down geographical barriers to care. Contributions illustrated the role of telemedicine telemedicine’s role in facilitating remote patient monitoring, virtual consultations, and continuous care delivery, especially in underserved areas. Telemedicine can also trigger more easily remote multidisciplinary care for patient with cardiovascular. This was particularly relevant in the current global health climate, where access to in-person healthcare services has been challenged.

Wearable devices and mobile health technologies were identified as key drivers in shifting the focus from treatment to prevention. By enabling continuous monitoring of vital signs and cardiac health indicators, these devices empower patients to play an active role in managing their health. This proactive approach highlights the potential for wearable technologies to alert patients and healthcare providers to early signs of CVD, facilitating timely intervention. Specialists in laboratory medicine should be actively engage in the validation and evaluation of this new generation of wearables and sensors.

The potential of 3D printing in cardiology was another significant perspective. It has revolutionized preoperative planning and patient education by allowing for the creation of patient-specific anatomical models. This technology enhances surgical precision and patient understanding of their condition, leading to improved surgical outcomes and patient satisfaction. Even if in the opinions received 3D printing directly relates to surgical applications, its integration within the lab setting for creating diagnostic tools and educational materials justifies its inclusion.

The figure illustrates these game-changing technologies in cardiovascular care

Figure 1: Game-Changing Technologies Redefining Cardiovascular Care.



Lastly, the project underscored the importance of equitable access to these emerging technologies. Despite the promising advancements, disparities in technology access and healthcare delivery remain a critical challenge. Contributors emphasized the need for policies and frameworks that ensure all patients, regardless of geographical or socio-economic status, benefit from these innovations.

The collective insights gleaned from the "Light After Armageddon" initiative not only underscore the transformative potential of emerging technologies in cardiovascular care but also chart a course for navigating the complexities of their integration into existing healthcare frameworks. As we distilled the wealth of contributions from experts across the spectrum of cardiovascular health, a multifaceted narrative emerges—one that balances the optimism of technological advancements with the pragmatism required for their effective deployment.

Central to this discussion is the acknowledgment of artificial intelligence (AI) as a linchpin in the evolution of cardiovascular diagnostics and treatment. AI's capacity to sift through and make sense of vast amounts of data heralds a new era of precision medicine, where treatments are not just patient-centred but are intricately tailored to the individual's unique physiological and genetic makeup.

Telemedicine and wearable devices stand out as technologies that democratize access to cardiovascular care, bridging the divide between remote or underserved populations and high-quality healthcare services. The pandemic has accelerated the adoption of these technologies, revealing a path forward where healthcare can be both ubiquitous and tailored. Yet, the widespread adoption of telemedicine and wearable technologies necessitates a robust digital infrastructure, underpinned by policies that protect patient data privacy while enabling seamless data sharing between patients and healthcare providers.

The advent of 3D printing in cardiology, offering personalized preoperative planning and education, exemplifies the tangible benefits of emerging technologies. However, to harness these benefits broadly, healthcare systems must navigate the challenges of cost, accessibility, and clinician training in the use of such advanced technologies.

Equity in access to these innovations emerges as a critical theme in the discourse on the future of cardiovascular care. The contributions highlight a significant gap between the promise of technological advancements and their accessibility to all segments of the population. Addressing this gap requires concerted efforts from policymakers, healthcare providers, and the tech community to devise strategies that ensure these life-saving technologies benefit everyone, irrespective of their socio-economic status or geographic location.

In conclusion, the "Light After Armageddon" initiative underscores a future where emerging technologies could potentially redefine cardiovascular care. However, realizing this future demands a collaborative approach that considers the ethical, logistical, and economic facets of technology integration into healthcare. It calls for a paradigm shift towards a more resilient, equitable, and patient-centric healthcare ecosystem, where the advancements heralded by our current technological renaissance are within reach of every heart that beats. As a key element of the diagnosis and monitoring of cardiovascular

diseases, specialists in laboratory medicine and clinical laboratories will be important parts and players for this future. As Charles Darwin aptly noted, “It is not the strongest of the species that survive, nor the most intelligent, but the one most responsive to change.”

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Research Article

Validation of the KL6 method on the G600II analyser (Lumipulse) for clinical use in interstitial lung disease

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Keywords

Laboratory method, validation, linearity, precisión, KL-6

Abstract

The Clinical Laboratory (CL) is involved in the prevention, diagnosis and follow-up of disease, as well as in the monitoring of treatment. For this reason, the CL must have robust quality systems in place in order to provide reliable results that help to ensure correct health care. Since the entry into force of the European regulation (IVDR) on in vitro diagnostic medical devices (EU) 2017/746 has generated the loss of CE marking in some laboratory determinations. In our case, Krebs von den Lungen-6 (KL-6), a diagnostic, severity and prognostic marker, as well as a marker of response to treatment, currently has the RUO (research use only) marking and, given its importance in our healthcare environment, we have validated the method with the new reagent in order to be able to continue with the clinical care of patients. In addition, this would keep this analyte within the scope of accreditation. Following the specific CLSI protocols, we carried out a study of precision, linearity as well as the limit of blank and the limit of detection, obtaining results within the limits established by the laboratory. This positive validation of KL6 allows us to continue using this analyte for clinical use and within the scope of accreditation.

Introduction

Lippi and Plebani define Laboratory Medicine as the discipline that deals with the quantitative measurement or qualitative evaluation of any substance in any biological fluid, for diagnostic or research purposes [1]. The results of the measurements obtained are intended to improve the care and/or well-being of the individual and the population. Thus, the Clinical Laboratory (CL) is involved in all aspects of patient care, i.e. from disease prevention, through diagnosis and follow-up, to treatment monitoring. This transversal perspective makes the CL a strategic point in the provision of healthcare, which, together with the growing technological evolution of measurement systems and the involvement of the CL in the diagnostic team, creates the need to review and update the multiple systems used for this purpose [2].

This fundamental task of the CL creates the need to establish robust quality management systems in which the measurement of analytes, applied knowledge and the competencies of the CL staff add value to healthcare by reducing potential laboratory errors and adapting demand management. The ISO 15189:2022 standard applies to all clinical laboratories, including those providing diagnostic, therapeutic and public health services. The aim of this standard is to promote patient

well-being through the quality and competence of clinical laboratories. In order to establish the management of a quality system in the CL, the standard indicates the obligatory nature of the procedures to be applied and the aspects to be taken into account in each of them, but does not specify how to establish them. Responsibility for quality is therefore left to the CL staff, based on knowledge of both the measurement method and the characteristics of the analyte together with the application and clinical repercussions. These quality management systems are dynamic, adapting to the changes that occur in the CL, either internally or externally [3-4]. The process of validating a method or assay involves providing objective evidence indicating compliance with the requirements for the previously defined analytical application. Typically, for in *vitro* diagnostic (IVD) methods, the supplier provides this information and the CL performs a verification of the method to ensure compliance with these requirements within the scope of its population and under its working conditions. Validation of the procedure lies with the CL only when it is a proprietary method or a method exclusively approved for research use only (RUO) [5].

Recently, a new European regulation (IVDR) on in *vitro* diagnostic medical devices, (EU) 2017/746, has come into force, which has meant that some tests which until now had CE marking have not been adapted to this new regulation and can only be used as RUO. This means that if a CL considers its continued use necessary for healthcare purposes, the CL itself will have to carry out this validation process. For this purpose, the Clinical and Laboratory Standards Institute (CLSI) has developed standardized evaluation protocol (EP) reference documents that help CLs to carry out these processes.

Krebs von den Lungen-6 (KL-6), also known as human mucin-1 (MUC-1), is a glycoprotein antigen with a high sialic acid content that is primarily expressed in type II pneumocytes. Due to its high molecular weight, its appearance in the bloodstream results from the destruction of the alveolar epithelium and/or increased capillary permeability [6-8]. Therefore, its blood levels are significantly increased in interstitial lung disease (ILD), a clinical condition characterized by the destruction of lung tissue with inflammation and fibrosis, in contrast to the healthy population and patients with other non-interstitial, non-fibrotic lung diseases or pneumonia [9-10]. Currently, the measurement of KL6, in combination with respiratory function tests and imaging techniques, has been proposed as a diagnostic, severity and prognostic marker [11-12], as well as a marker of treatment response [13-14].

Recently, our supplier of the KL-6 reagent has changed the CE-approved marking to RUO, without any change in the manufacture of the reagent. This change in the marking of the KL-6 reagent triggered the need for our CL to validate the method with the new reagent, in order to continue with the clinical care of patients with interstitial pneumonia, both in the initial diagnosis and in the follow-up of this disease and the potential complications derived from connective tissue diseases. In addition, this would enable the analyte to remain within the scope of accreditation.

Material and methods

The KL-6 validation study was conducted during August 2023 in a tertiary hospital in the Community of Madrid, Spain.

The supplier Fujirebio Europe NV provided Lumipulse® G KL-6 reagent (reference 234594), Lumipulse® PIVKA-II and KL-6 Controls (reference 233900) and Lumipulse® G KL-6 calibrators (Reference 234600).

The validation was performed on the automated platform LUMIPULSE® G600II (Fujirebio) with serial number KF150111B.

The method of determination is a sandwich-type chemiluminescence enzyme immunoassay (CLEIA).

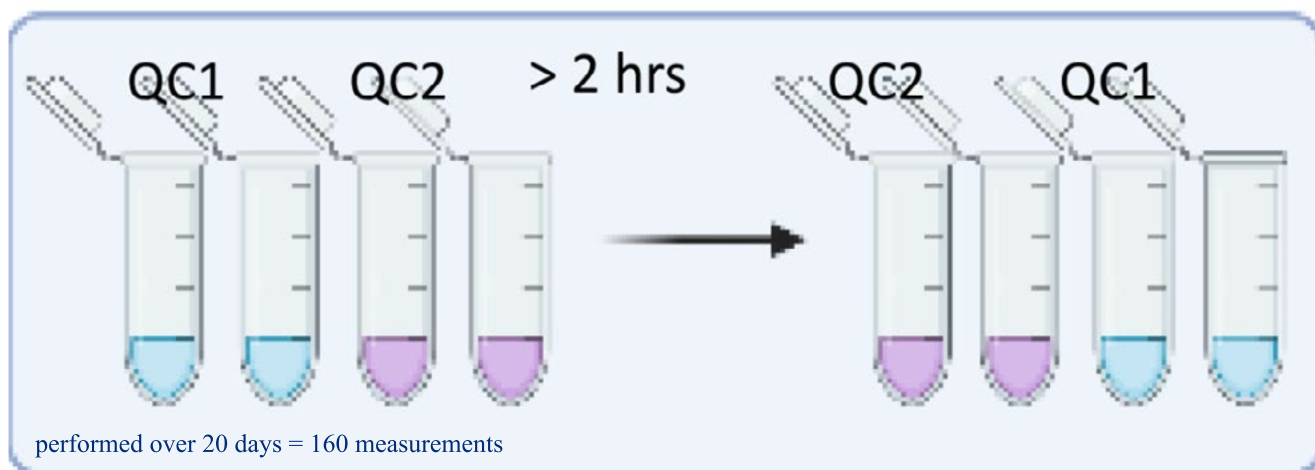
A method validation plan was carried out which included evaluation of precision, linearity, limit of blank and limit of detection of the technique for the RUO-labelled KL-6 reagent, following the relevant Clinical and Laboratory Standards Institute (CLSI) protocols.

Clinical validation in patients was rejected due to previous experience with the analyte, and the CL staff being familiar with its behavior and clinical utility.

1. Evaluation of precision

The CLSI EP05-A3 protocol “Evaluation of Precision of Quantitative Measurement Procedures” [15] was followed. The level 1 control (QC1) and level 2 control (QC2) were analyzed for 20 consecutive days in duplicate with a concentration of 328 and 844 U/mL respectively. This series was repeated twice a day with a time interval of at least 2 hours (Figure 1). The decision limit for total error was set as medical relevance at 10%, as this was the value previously established in our daily quality assurance practice.

Figure 1: Evaluation of precision.



Measurement of the two control levels in duplicate. Repeat this series again after two hours. This is repeated for 20 days for a total of 160 determinations.

2. Evaluation of linearity

The CLSI EP06 protocol “Evaluation of Linearity of Quantitative Measurement Procedures” [16] was followed. Nine concentration levels were evaluated: 44 IU/mL, 346 IU/mL, 698 IU/mL, 951 IU/mL, 1294 IU/mL, 1621 IU/mL, 1954 IU/mL, 2272 IU/mL and 2496 IU/mL. These concentrations were obtained from a pool of patient samples to obtain the lowest and highest concentrations, 44 IU/mL and 2496 IU/mL, respectively. Using these initial concentrations, the above-mentioned protocol was followed by performing the dilutions shown in Table 1 below. The analyte was then analyzed in triplicate for each concentration level in a single test run.

Table 1: Concentrations obtained from the sample pool.

Theoretical concentration (IU/mL)	Sample quantity low concentration (mL)	Sample quantity high concentration (mL)
44 (Low)	1	0
346	0.875	0.125
698	0.750	0.250
951	0.625	0.375
1294	0.500	0.500
1621	0.375	0.625
1954	0.250	0.750
2272	0.125	0.875
2496 (High)	0	1

The different dilutions carried out to obtain the theoretical concentrations

3. Evaluation of limits of blank and detection

The CLSI EP17-A2 protocol “Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures” [17] was followed.

The limit of blank (LoB) is defined as the highest apparent concentration of the analyte when replicas of a blank sample without analyte are measured. It refers to the signal/noise of the analyzer and not to the actual concentration of the analyte. For evaluation of the LoB, 60 measurements of the analyte were performed using the zero calibrator as the sample.

The limit of detection (LoD) is the lowest concentration of analyte detectable at a given confidence level, and therefore a sample of known concentration of 26 IU/mL was used for the evaluation of this limit, and measured 60 times (Figure 2).

Figure 2: Evaluation of limit of blank and detection.

3 ST0 tubes x 20 repetitions/1 day = 60 measurements

3 sample tubes 26 IU/mL x 20 repetitions/1 day = 60 measurements

Measurement of three aliquots of calibrator 0 repeated 20 times in one day for a total of 60 determinations. The same series is performed with a sample concentration of 26 IU/mL.

4. Statistical methods

After performing the necessary procedures included in the validation plan, the results obtained were analyzed together with the Quality Department of the Clinical Analysis Service and a report was issued for each result indicating whether the analyte met the previously established acceptance criteria, using the Analyse-it v6.15 program.

The decision limit for total error was set as medical relevance at 10%, as this was the value previously established in our daily quality assurance practice.

Results

Evaluation of precision. The results obtained from the analysis are within the precision limits established by the laboratory of 10% at the two concentration levels studied, 328 IU/mL and 844 IU/mL, with a coefficient of variation of 3.4% and 2.7% respectively.

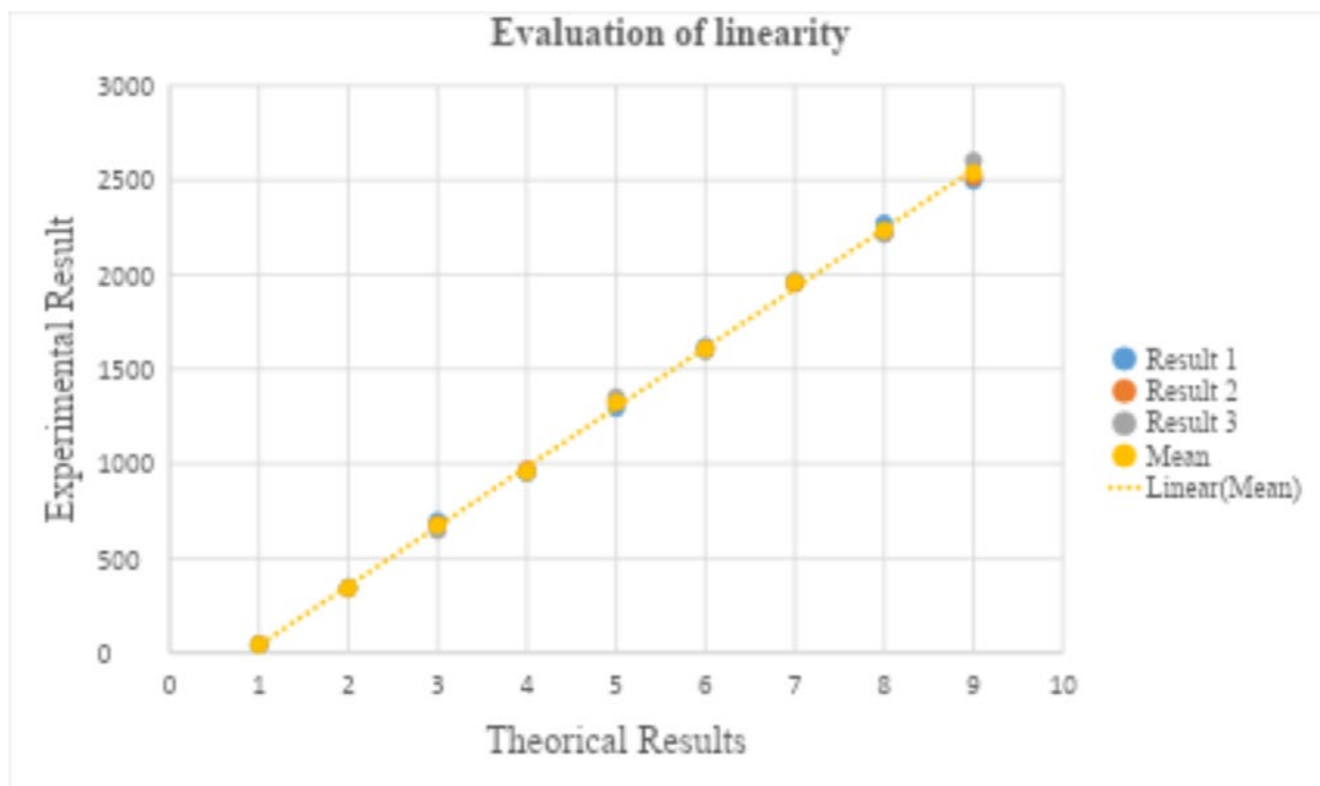
Table 2: Results of evaluation of precision.

Precision				
Theoretical concentration (IU/mL)	**Average concentration (IU/mL)**	**Coefficient of variation (CV)**	**Standard deviation (SD)**	**Allowable SD/CV**
328	355.3	3.4 %	12.2	10%
844	913.2	2.7 %	24.6	10%

The CVs and SDs obtained at the two concentrations at which the accuracy was studied.

Evaluation of linearity range. The results obtained from the analysis, setting the precision limit at 10%, indicate that the method studied is linear from a concentration of 44 IU/mL to 2496 IU/mL (Figure 3). Samples with a concentration below 44 IU/mL shall be reported as <44 IU/mL and samples with a concentration above 2496 IU/mL shall be diluted (Table 3).

Figure 3: Evaluation of linearity.



Graph generated from the results obtained after the evaluation of linearity.

Table 3: Results of the evaluation of linearity.

Theoretical result	Mean	Linear fit	Nonlinearity	90% familywise CI (98,89% individual CI)	Allowable nonlinearity
44.000	43.0	39.1	10.0*	-87.6% to 107.6%	±10.0%
359.625	341.7	353.7	-3.4%	-14.2% to 7.4%	±10.0%
675.250	674.0	668.4	0.8%	-4.9% to 6.5%	±10.0%
990.875	957.3	983.0	-2.6%	-6.5% to 1.3%	±10.0%
1306.500	1324.0	1297.7	2.0%	-0.9% to 5.0%	±10.0%
1622.125	1607.3	1612.3	-0.3%	-2.7% to 2.1%	±10.0%
1937.750	1959.0	1927.0	1.7%	-0.3% to 3.6%	±10.0%
2253.375	2235.0	2241.6	-0.3%	-2.0% to 1.4%	±10.0%
2569.000	2537.7	2556.2	-0.7%	-2.2% to 0.8%	±10.0%

*Performance requirement not met
Results obtained in the evaluation of linearity at each concentration level studied

Evaluation of limit of blank and detection. The results obtained from the analysis showed that the limit of quantification is 26 IU/mL with imprecision of 3.7%, enabling differentiation of the concentration of the samples from the zero concentration with a coefficient of variation of 5.2% and reporting of patient results as <26 IU/mL, as shown in Table 4.

Table 4: Results of the absorbances.

Precision			
Theoretical concentration (IU/mL)	**Mean absorbance**	**Coefficient of variation (CV)**	**Standard deviation (SD)**
0	720.8	5.2 %	37.3
26	10400.3	3.7 %	381.7

Results of the absorbances obtained at theoretical concentrations of 0 and 26 IU/mL.

Discussion

Occasionally, changes in the internal policies of the suppliers of the reagents used in the CL lead to changes in the activity of the laboratory staff. In our case, removal of the CE marking from the KL-6 reagent and its switch to RUO required the CL staff to evaluate the requirements and specifications necessary to validate the KL-6 method and thus continue with the clinical care of patients treated in our healthcare area.

After evaluation of the results obtained from the precision study, it can be said that the KL-6 measurement does not exceed the limit of precision of 10% established by the laboratory for the two concentration levels studied, 328 and 844 (IU/mL), and therefore complies with the CL's quality assurance. Furthermore, based on the results of the limit of blank and detection evaluation, it can be established that the reagent used in the LUMIPULSE platform (Fujirebio) is able to differentiate the background noise of the analyzer from the concentration of the analyte and to measure a KL-6 concentration of 26 IU/mL with a coefficient of variation of less than 10%, specifically 3.7%.

Regarding the linearity range of the technique, we studied the concentration range from 44 IU/mL to 2496 IU/mL, and were able to establish that it is linear in this range. Thus, concentrations below 44 IU/mL should be reported as <44 IU/mL and concentrations above 2496 IU/mL should be diluted. The choice of this range arose for three fundamental reasons, firstly because of the availability in the CL of the pool of serum samples from patients with these concentrations, secondly because the dilution recommendations of the CLSI protocol for the linearity range should be followed, and finally because the clinical decision level of KL-6 for healthy versus pathological discrimination was known and established as 500 IU/mL according to the literature. It is worth noting that although we know that at KL-6 concentrations of 26 IU/mL the inaccuracy is less than 10%, we report KL-6 results as less than 44 IU/mL because we have studied linearity in the range 44-2496 IU/mL and we do not know if it meets linearity criteria between the concentrations of 26-44 IU/mL. In addition, the disease associated with this analyte is produced by elevation of its concentration, with no clinical repercussions at the previously mentioned concentration levels of 26 or 44 IU/mL.

Another point that we consider important in our laboratory is the loss of ISO 15189:2022 accreditation of KL-6, due to the RUO marking of this reagent. After this validation, which covers the different analytical quality aspects required for the accreditation of this test, in the next external audit we will declare the evaluation of this analyte in order to obtain its accreditation.

Conclusions

In conclusion, it can be stated that the RUO-labelled KL-6 reagent measured on the LUMIPULSE platform (Fujirebio) meets the quality assurance criteria established in our laboratory and can be used in routine clinical practice, although it does not have the IVDR marking.

Having carried out an experimental design following CLSI protocols means that the results obtained could be useful for other clinical laboratories interested in incorporating this analytical method into their healthcare service portfolio.

Limitations

An assessment of the range of linearity between the KL-6 concentration of 26 IU/mL and 44 IU/mL would be necessary in order to report patient results as below 26 IU/mL, although at these levels there is no clinical impact and therefore patient management does not change.

Declaration of Conflict of interests

The authors of this article declare that there is no conflict of interest with regard to the content of this manuscript.

Ethical Considerations

This study did not involve patients and therefore no declaration of ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki, is required.

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Review Article

Vitamin D Controversies in the Laboratory Medicine: A Review of Clinical Guidelines and Recommendations

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Keywords
clinical practice guideline, deficiency, position statement, recommendations, reference values, vitamin D.

Abstract

A narrative review of the main guidelines and recommendations published from 2011 up to date about the status of vitamin D deficiency has been carried out. The objective of this review is to discuss the origin of the controversy about the status of this entity, as well as the evolution of the methodological aspects and clinical situations that require vitamin D screening.
The results obtained indicate that the criteria defining vitamin D status, according to two studies published in 2011, the Institute of Medicine (IOM) recommendations and the Endocrine Society (ES) guidelines, regardless the affected population.
Concerning the methodology used, progress has been made thanks to the Vitamin D Standardization Program (VDSP), although the most recent results from the external Vitamin D External Quality Program Assessment Scheme (DEQAS) indicate that there is still a significant bias among the different immunoassays available.
In relation to the criteria for screening, an agreement is observed in the most recent publications.

Introduction

Vitamin D remains to be a controversial issue for several reasons: the lack of consensus to define vitamin D status [1], the great rise of publications that relate the concentration of 25-hydroxyvitamin D (25-(OH)D) to different pathophysiological situations without enough evidence [2], the analytical variability derived from the various

methodologies [3], and the lack of consensus among scientific societies and governmental health institutions in countries in which the refundability of vitamin D supplements depends on the definition of hypovitaminosis or the quantification of baseline vitamin D levels according to clinical diagnosis [4]. All these reasons are causing an increase in the measurement of 25-(OH) D in clinical laboratories [5,6], in the number of supplemented patients [7], and the need to agree on decision values in reports [4].

In order to explain the current situation, it is necessary to understand how the main vitamin D guidelines and recommendations have evolved. In 1991, the United Kingdom Nutrition Committee (COMA) established for first time that plasma levels of 25-(OH)D below 8 ng/mL were present in children with rickets [8]. However, it was not until 2011 when the main aspects responsible for establishing nutritional recommendations for vitamin D emerged, and, therefore, the reference intervals of plasma concentrations associated to the nutritional status of the population appeared.

Two of these aspects, described by the COMA (updated in 2016) [9] and the Institute of Medicine (IOM) with the study of the population of USA and Canada [10], agree in defining the deficiency status. However, the IOM expands the states to insufficiency, sufficiency, and toxicity.

In 2011, the clinical practice guideline on vitamin D of the Endocrinology Society was published [11], presenting notable differences from the two previous approaches regarding the definition of vitamin D status. The reason for this discrepancy may be that this latest guideline is based on the vitamin D recommendations of the International Osteoporosis Foundation (IOF), established on the basis of randomized clinical trials in the adult population [12].

The controversy generated in the scientific community by this latest guideline was such that, from 2017 to 2019, three international conferences were held in Italy to discuss topics related to the definition of vitamin D status and methodological aspects of the quantification of 25-(OH)D plasma levels [13-15], reaching the conclusion of the need for standardization of the methodology in order to achieve consensus in the definition of vitamin D status.

The Vitamin D Standardization Program (VDSP) was founded in 2010. As a result of the tools developed by the VDSP, currently there are a reference method, standard reference materials (SRMs), quality standards based on biological variability for both reference and routine laboratories, and external quality assurance criteria that programs must meet.

Currently, only two quality assurance programs meet the VDSP requirements, the one of the College of American Pathologists (CAP) and the Vitamin D External Quality Assessment Scheme (DEQAS). In this programs, target values are assigned to each serum sample using the NIST (The National Institute of Standards and Technology) or CDC (Centers for Disease Control and Prevention) Reference Measurement Procedure (RMP), and participants' performance of specific methods for 25-(OH)D and other vitamin D metabolites are assessed [16]. In this regard, a recent DEQAS publication shows the analytical variability of the main current methods for measuring the concentration of 25-(OH)D [17].

The objective of this study is to review the definitions of vitamin D status in the main guidelines and recommendations on the main scientific databases, as well as the current state of the methodology available for its quantification.

Material and Methods

Over the last decade, the number of vitamin D-related publications has dramatically increased. Therefore, we decided to focus on the largest and most relevant guidelines, recommendations, and position statements to define vitamin D status, as well as on recent studies of our interest to analyze methodological quality.

We established a time period from January 2011 to December 2023. In case of more than one review being published by the same scientific entity throughout this period of time, the latest one was considered.

Search strategy

A strategic search was carried out using several electronic databases: Medline/PubMed, Web of Science, and Scopus; and looking for combinations of the following search terms: vitamin D, deficiency, nutrition, references values, dietary references, 25-(OH)D measurement, clinical practice guideline, recommendations, and position statement. Studies not written in English or Spanish were excluded.

Results

A total of 40 issues that establish vitamin D status, 9 clinical guidelines and 31 recommendations of population studies supported by relevant scientific organizations and/or committees have been reviewed.

The main aspects related to the clinical laboratory that determine the vitamin D status since 2011 are summarized in Table 1. It is based on the three main documents published up to date: the clinical practice guideline of the ES of 2011, the recommendations of the IOM of 2011, and the recommendations of the Scientific Advisory Committee on Nutrition (SACN) of 2016. Table 1 shows laboratory advices to establish vitamin D status, as 25-(OH)D cutoff points, reference intervals according to the type of requirements, the need for screening, and the methodology recommended for the measurement of 25-(OH)D [9-11].

Table 1: Comparison of the recommendations of the Endocrine Society, the Institute of Medicine and the Scientific Advisory Committee on Nutrition about the optimal concentration of 25-(OH) vitamin D.

	SACN. Vitamin D and Health, 2016 [9]	A. Catharine Ross et al. Dietary Reference Intakes for Calcium and Vitamin D. IOM, 2011 [10]	**Holick et al. Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an ES Clinical Practice Guideline, 2011 [11]**
Serum 25-(OH)D cutpoints	Serum 25-(OH)D concentration is an indicator of exposure to vitamin D (from skin synthesis and dietary intake). • 25 nmol/L (10 ng/mL) In order to protect musculoskeletal health, it is recommended that serum 25-(OH)D concentration in all individuals in the UK should not fall below 25 nmol/L at any time of the year. • <30 nmol/L (<12 ng/mL) A serum 25-(OH)D concentration <30 nmol/L was associated with: increased risk of rickets, impaired fractional calcium absorption and increased risk of osteomalacia in young and middle-aged adults, and impaired fractional calcium absorption and fracture risk in older adults. A serum concentration of 30 nmol/L was considered to be consistent with the lower end of requirements. • 50 nmol/L (20 ng/mL) It was also concluded that there was a trend for maximal calcium absorption at serum concentration of 50 nmol/L. 50 nmol/L would cover the needs of most individuals in terms of vitamin D and this was used to establish the RDAs intake value for vitamin D. Little causal evidence for additional benefits on BMD, fracture risk or osteomalacia risk at serum 25-(OH)D concentration >50 nmol/L.	< 30 nmol/L (< 12 ng/mL) = deficiency 30 – 50 nmol/L (12 – 20 ng/mL) = Inadequacy, but not for all persons > 50 nmol/L (> 20 ng/mL) = Sufficient level > 75 nmol/L (> 30 ng/mL) = not associated with increased benefit The committee noted with some concern that serum 25-(OH)D cut-points defined as indicative of deficiency for vitamin D have not undergone a systematic, evidence-based development process.	< 50 nmol/L (< 20 ng/mL) = deficiency 50 – 73 nmol/L (20 – 29 ng/mL) = insufficiency 75 – 250 nmol/L (30 – 100 ng/mL) = Sufficient level

Screening	Not provided	Not evaluated. This committee considers that the evidence surrounding bone health provides a reasonable and supportable basis to allow the vitamin D to be used for DRIs development.	There is not sufficient evidence to recommend screening individuals who are not at risk for deficiency. Candidates for screening: rickets, osteomalacia, osteoporosis, chronic kidney disease, hepatic failure, malabsorption syndromes, hyperparathyroidism, some medications, African-American and Hispanic children and adults, pregnant and lactating women, older adults with history of falls, older adults with history of nontraumatic fractures, obese children and adults, granuloma-forming disorders, some lymphomas.
Assays for 25-(OH)D levels	Quantification of serum 25-(OH)D concentration can vary considerably (15-20%) depending on the type of assay used and across different concentration ranges.	There are differences in assay methodologies used. Reports in the literature for serum 25-(OH) D measures should be carefully interpreted, taking into account the type of assay employed, use of automation, year of analysis, and context of the analysis.	All clinical assays, including 25-(OH)D measurements, are subject to variability. Such variability confounds attempts to define a single "cut point" value as indicating low vitamin D status. For clinical care, all current methodologies seem adequate if they target 25-(OH)D values higher than current cut points.
Rickets and Osteomalacia	Evidence on vitamin D and rickets is mainly observational. Individual and mean serum 25-(OH)D concentrations of children with rickets were < 25 nmol/L (<10 ng/mL) in the majority of studies. Evidence on osteomalacia is limited mainly to case reports in which serum 25-(OH)D concentrations ranged between 4 and 20 nmol/L (1.6-8 ng/mL).	Serum 25-(OH)D levels lower than 27 to 30 nmol/L (10 to 12 ng/mL) are not diagnostic but associated with an increased risk for developing rickets. The risk of rickets increases below a serum 25-(OH)D level of 30 nmol/L (< 12 ng/mL) and is minimal when serum 25-(OH) D levels range between 30 - 50 nmol/L (12-20 ng/mL). Moreover, when calcium intake is inadequate, vitamin D supplementation to the point of serum 25-(OH)D concentrations up to and beyond 75 nmol/L (30 ng/mL) has no effect.	All available evidence suggests that children and adults should maintain a blood level of 25-(OH)D above 20 ng/ml to prevent rickets and osteomalacia, respectively. However, to maximize vitamin D's effect on calcium, bone, and muscle metabolism, the 25-(OH)D blood level should be above 30 ng/ml.

Falls and fractures	Evidence on vitamin D and falls is mixed but, overall, was suggestive of a beneficial effect of vitamin D supplementation in reducing fall risk in adults ≥ 50y with mean baseline serum 25-(OH)D concentrations ranging between < 25 and around 80 nmol/L (<10-32 ng/mL).	Some studies identified specific serum concentrations of 25-(OH)D below which falls, fractures, or bone loss increased; these values ranged from approximately 40 to 80 nmol/L. (16 – 32 ng/mL). Although some studies suggested that serum 25-(OH)D concentrations of approximately 40 nmol/L (16 ng/mL) are sufficient to meet bone health requirements for most people, findings from other studies suggested that levels of 50 nmol/L and higher (> 20 ng/mL) were consistent with bone health.	25-(OH)D between 30 and 40 ng/ml are consistent with the threshold for hip and nonvertebral fracture prevention from a recent meta-analysis of double-blind randomized controlled trials (RCT) with oral vitamin D.
Non-musculoskeletal health outcomes	There are insufficient data to draw conclusions on the relationship between serum 25-(OH)D concentration and non-musculoskeletal health outcomes	Outcomes related to cancer/neoplasms, cardiovascular disease and hypertension, diabetes and metabolic syndrome, falls and physical performance, immune functioning and autoimmune disorders, infections, neuropsychological functioning, and preeclampsia could not be reliably linked with calcium or vitamin D intake and were often conflicting.	Numerous studies have demonstrated an association of vitamin D deficiency with increased risk of more than a dozen cancers; autoimmune diseases, including both type 1 and type 2 diabetes, rheumatoid arthritis, Crohn's disease, and multiple sclerosis; infectious diseases; and cardiovascular disease. There are, however, very few RCT with a dosing range adequate to provide evidence for the benefit of vitamin D in reducing the risk of these chronic diseases

25-(OH)D: 25-hydroxyvitamin D; BMD: bone mineral density; DRIs: dietary reference intakes; ES: Endocrine Society; IOM: Institute of Medicine; RDAs: recommended dietary allowances; SACN: Scientific Advisory Committee on Nutrition.

It is remarkable that the clinical practice guideline of the ES from 2011 principally disagrees on the definition of vitamin D deficiency and sufficiency, and propose higher cutoff points as reference than those published in population studies carried out in the US, Canada, and UK: for ES the deficiency status is < 50 nmo/L, while for IOM and SACN it is at levels < 25-30 nmol/L, and for ES the sufficiency status is between 75-250 nmol/L, while for IOM and SACN sufficiency is reached at levels 50 nmol/L, with no evidence of benefit above 75 nmol/L. This means that ES differs in the preventive values for bone and musculoskeletal health (rickets, osteomalacia, fractures, and falls), establishing concentrations between 10-20 ng/mL higher than the recommendations of IOM and SACN (falls and fractures: for IOM and SACN prevention is from 20-32 ng/mL, while for ES it is from 30-40 ng/mL; rickets and osteomalacia: for IOM prevention is from 12-20 ng/mL, while for ES it is from 20 ng/mL). Nevertheless, the three main studies agree on the methodological variability for the determination of 25-(OH)D.

The main guidelines and recommendations that have emerged subsequently and up to date are listed in Table 2, consisting of 8 clinical guidelines [24, 29, 32, 36, 37, 41, 49, 54] (3 of them related to bone health [32,41,49]), and 29 studies related to recommendations on vitamin D status [18-54]. Table 2 shows information of interest to clinical laboratories, such as the ranges to define vitamin D status, methodological aspects recommended, the need for population screening, and similarity with the main previous publications.

Depending on the tendency followed, the definition of vitamin D status may differ. In this sense, 13 studies apply the IOM recommendations, 11 studies take into consideration the recommendations of the ES, 8 studies collect information from both aspects, and only 3 studies consider the recommendations of the SACN. Among 2019 and 2023 there has been a trend in taking into consideration from both IOM [10] and ES [11].

Table 2: Status of 25-(OH)D levels according different guidelines, position statement and recommendations, and consistency with ES 2011, IOF 2010, IOM 2011 or SACN 2016.

Clinical guideline/ Position Statement/ Recommendation, year	**Status of vitamin D and 25(OH) D concentration**	**Information about laboratory assay**	**Measurement of 25 (OH)D as screening test and recommended testing in:**	**Consistent with:**
New Reference Values for Vitamin D, German Nutrition Society, 2012 [18]	Serum 25-(OH)D concentrations of 50 nmol/L (20 ng/mL) or higher are considered an indicator of optimal vitamin D status. Currently, 30 nmol/l (12 ng/ mL) is the concentration that is deemed necessary for reliable rickets prophylaxis.	Not provided	Not provided	IOM 2011 IOF 2010
Vitamin D and health in adults in Australia and New Zealand: a position statement, 2012 [19]	Vitamin D adequacy: ≥ 50 nmol/L (≥ 20 ng/mL) at the end of winter (10–20 nmol/L higher at the end of summer). Mild vitamin D deficiency: 30–49 nmol/L (12-19 ng/mL). Moderate vitamin deficiency: 12.5–29 nmol/L (5-11 ng/mL). Severe vitamin D deficiency: < 12.5 nmol/L (< 5 ng/mL).	The bias and imprecision of many automated methods may be problematic at the lower, clinically and analytically important range (< 50 nmol/L) of the assay. Some laboratories are using more precise methods of analysis, such as LC-MS/ MS	Screening in groups at high risk for vitamin D deficiency: people with a disability or chronic diseases, fair-skinned people and those at risk of skin cancer who avoid sun exposure, obese people, people working in an enclosed environment. In some high-risk groups (dark-skinned migrants, people in residential care establishments) screening test it is not necessary.	IOM 2011
British Paediatric and Adolescent Bone Group's position statement on vitamin D deficiency [20]	Deficiency: < 25 nmol/L 25-(OH)D (< 10 ng/mL). Insufficiency: 25-50 nmol/L 25-(OH)D (10-20 ng/mL). Sufficiency: > 50 nmol/L 25-(OH) D (> 20 ng/mL).	Not provided	Not provided	IOM 2011
Vitamin D: Still a topical matter in children and adolescents. A position paper by the Committee on Nutrition of the French Society of Paediatrics, 2012 [21]	The normal range was defined by the mean +/- 2 SD of the 25-(OH) D value sampled in a population of healthy subjects, i.e., 25 to 137.5 nmol/L (10-55 ng/mL) for European and North American populations.	The measurement method must be reliable and take into account the 2 fractions: 25-(OH)D2 and 25-(OH) D3. Laboratories must use external quality assurance programs such as the DEQAS international control system.	Not provided	IOM 2011
Recommended intake of calcium and vitamin D: positioning of the Nutrition Committee of the AEP, 2012 [22]	In adults, an indirect correlation between 25(OH)D and PTH levels permit accepting the deficiency cutoff point at 50 nmol/L (20 ng/ ml). This level tends to apply to children of any age.	Lack of standardization of measurement methods.	Not provided	IOM 2011

Evaluation of dietary reference values for vitamin D, Health Council of the Netherlands, 2012 [23]	25-(OH)D ≥ 30 nmol/L (≥ 12 ng/mL) all the year for people aged between 4 and 70, including lactating women, and ≥ 50 nmol/L (≥ 20 ng/mL) in subjects above 70 years.	Serum 25-(OH)D concentration is associated with a CV of 15 - 20%, due to variations in analytical methods.	Not provided	IOM 2011
Guideline: Vitamin D supplementation in pregnant women. World Health Organization, 2012 [24]	IOM determined serum levels of 25-(OH)D > 50 nmol/L (> 20 ng/mL) as adequate for pregnant women. However, other experts argue that optimal levels should be >75 nmol/L (> 30 ng/mL).	Not provided	Not provided	IOM 2011 ES 2011
Vitamin D deficiency: Evidence, safety, and recommendations for the Swiss population. Expert report for the FCN, 2012 [25]	Vitamin D deficiency: < 50 nmol/L (< 20 ng/mL) Severe Vitamin D deficiency: < 25 nmol/L (< 10 ng/mL) Vitamin D insufficiency: 25-49 nmol/L (10 to 19 ng/mL) Adequate Vitamin D threshold: ≥ 50 nmol/L (≥ 20 ng/mL) Desirable Vitamin D for fall and fracture reduction: 75-110 nmol/L (30 - 44 ng/mL).	Assay variability for 25-(OH)D measurement d epends on the methodologies used. Efforts to improve assay comparability are important using uniform standards available through the NIST.	Only in individuals at high risk for severe vitamin D deficiency: bone disorders, hyperparathyroidism, older adults with falls or low trauma fractures, obesity, pregnant and lactating women not taking vitamin D supplements, children and adults with a dark skin tone, athletes who primarily exercise indoors, chronic kidney/hepatic diseases, and malabsorption syndromes.	IOM 2010 IOF 2010 ES 2010
Nordic Nutrition Recommendations 2012. Integrating nutrition and physical activity. Nordic Council of Ministers [26]	A serum 25-(OH)D concentration of 50 nmol/L (20 ng/mL) is used as an indicator of sufficiency, and a concentration of 30–50 nmol/L (12-20 ng/mL) is considered to indicate insufficient status.	The VDSP has the aim of standardizing serum 25-(OH)D concentration measurements. Results from some immunoassay methods have shown lower 25-(OH)D values as compared to HPLC or LC-MS/MS (standard method proposed). This should be accounted for when interpreting results.	Not provided	IOM 2011

Vitamin D and health in pregnancy, infants, children and adolescents in Australia and New Zealand: a position statement. Australian and New Zealand Bone and Mineral Society; Osteoporosis Australia, 2013 [27]	Severe deficiency 25-(OH) D: <12.5 nmol/L (<5 ng/mL). Moderate deficiency 25-(OH)D: 12.5–29 nmol/L (5-11.6 ng/mL). Mild deficiency 25-(OH)D: 30–49nmol/L (12-19.6 ng/dL). Sufficient 25-(OH) D: ≥ 50 nmol/L (≥ 20 ng/mL) . Elevated 25-(OH) D: >250nmol/L (> 100 ng/mL). The recommended level for serum 25-(OH)D in infants, children, adolescents and during pregnancy and lactation is 50 nmol/L (20 ng/mL), and 10–20 nmol/L (4-8 ng/mL) higher at the end of summer.	There is a degree of imprecision in current testing (around 10%). Laboratories offering 25-(OH)D testing are required to participate in external quality assurances programs.	There is inadequate evidence to recommend population-wide screening for vitamin d status in infants, children and adolescents. Only in case of one or more risk factors for low vitamin D: lack of skin exposure to sunlight, dark skin, medical conditions or medication affecting vitamin D metabolism.	IOM 2011
Vitamin D in the Healthy European Paediatric Population, 2013 [28]	Sufficiency 25-(OH)D: > 50 nmol/L (> 20 ng/mL). Severe deficiency 25-(OH)D: < 25 nmol/L (< 10 ng/mL).	There are essential inter-assay differences in commercially available 25-(OH)D tests.	Not provided	IOM 2011 IOF 2010 ES 2011
Practical guidelines for the supplementation of vitamin D and the treatment of deficits in Central Europe-recommended vitamin D intakes in the general population and groups at risk of vitamin D deficiency, 2013 [29]	Deficiency 25-(OH)D: < 50 nmol/L (< 20 ng/mL). Suboptimal status 25-(OH)D: 50-75 nmol/L (20-30 ng/mL). Adequate status 25-(OH)D: 75 – 125 nmol/L (30-50 ng/mL). High vitamin D supply: 125-250 nmo/L (50-100 ng/mL). Risk for overall health outcomes: > 250 nmol/L (>100 ng/mL). Toxic status: > 500 nmol/L (> 200 ng/mL).	Methods must measure both 25-(OH)D2 and 25-(OH) D3. Intra-assay CV should be < 5%, and inter-assay CV < 10%.	Not provided	IOM 2011
Recommended Vitamin D intake and management of low Vitamin D status in adolescents: a position statement of the Society for Adolescent Health and Medicine, 2013 [30]	Deficiency 25-(OH)D: < 50 nmol/L (< 20 ng/mL). Insufficient status 25-(OH) D: 50-72.5 nmol/L (20-29 ng/mL). Normal vitamin D status: > 75 nmol/L (> 30 ng/mL). Optimal vitamin D status for adolescents: 75 – 125 nmol/L (30- 50 ng/mL). Toxic status 25-(OH)D: > 500 nmol/L (> 200 ng/mL).	Not provided	Testing in high risk of low vitamin D status: increased skin pigmentation, frequent use of sunscreen, obesity, specific diet, cultural convention associated with body coverage, malabsorption syndromes, amenorrhea, pregnancy or lactation, immobilization, bariatric surgery, chronic kidney/hepatic diseases, specific medication, recurrent fractures or low bone mineral density status.	ES 2011

Recommendations Abstracted from the American Geriatrics Society Consensus Statement on Vitamin D for Prevention of Falls and their consequences, 2014 [31]	A serum 25-(OH)D concentration of 75 nmol/L (30 ng/mL) should be a minimum goal to achieve in older adults, particularly in frail adults.	Not provided	In older adults only in situations of risk: hypercalcemia, individuals taking medications that bind to vitamin D or accelerate the breakdown, obesity, malabsorption syndromes, intake below recommended.	ES 2011
Clinician's Guide to Prevention and Treatment of Osteoporosis, 2014 [32]	Insufficiency: serum 25-(OH)D < 75 nmol/L (< 30 ng/mL).	Not provided	Not provided	IOF 2010
Optimizing Bone Health in Children and Adolescents, 2014 [33]	25-(OH)D reference interval for healthy children and adolescents: ≥ 50 nmol/L (≥ 20 ng/mL). 25-(OH)D reference interval for people at increased risk of fracture: ≥ 75 nmol/L (≥ 30 ng/mL).	Not provided	Evidence is insufficient to recommend universal screening. Screening only in children and adolescents with reduced bone mass and/or recurrent low-impact fractures.	IOM 2011
Dietary reference values for vitamin D. EFSA Panel on Dietetic Products, Nutrition and Allergies, 2016 [34]	For adults, infants and children there is evidence of an increased risk of adverse musculoskeletal health outcomes and adverse pregnancy-related health outcomes at serum 25-(OH)D concentration below 50 nmol/L (20 ng/mL).	The introduction of a NIST standard reference material for vitamin D has been a step forward in providing a reference measurement procedure against which assays could be standardized to avoid variability of results. Free serum 25-(OH)D and plasma/serum 1,25-(OH)2D concentration cannot be used as a biomarker of vitamin D status.	Not provided	IOM 2011 SACN 2016
Global consensus recommendations on prevention and management of nutritional rickets, 2016 [35]	Sufficiency 25-(OH) D: > 50 nmol/L (> 20 ng/mL). Insufficiency 25-(OH)D: 30-50 nmol/L (12-20 ng/mL). Deficiency 25-(OH) D: < 30 nmol/L (< 12 ng/mL). Toxicity 25-(OH)D: > 250 nmol/L (> 100 ng/mL).	The reliability of immunoassays is questioned particularly at low and high concentrations of 25-(OH) D. The reduction of the inter-laboratory variation in 25-(OH)D measurements are observed using HPLC-MS/MS with the application of NIST standard reference materials.	**Not provided**	IOM 2011

Clinical practice guidelines for vitamin D in the United Arab Emirates, 2016 [36]	Deficiency 25-(OH)D: < 50 nmol/l (< 20 ng/mL) . Insufficiency 25-(OH)D: < 75 nmol/L (< 30 ng/mL). Recommended 25-(OH)D level: 75 – 150 nmol/L (30-60 ng/mL).	All clinical assays are subject to significant assay variability. The comparability of 25-(OH) D results seems likely to improve as uniform standards (NIST).	Testing only in pretreatment and in situations of risk: bone disorders, abnormalities of calcium and/or phosphate metabolism, hyperparathyroidism, specific medication, malabsorption syndromes, eating disorders, chronic kidney/hepatic diseases, granulomatous disorders, cancer, cardiovascular diseases, metabolic syndrome, chronic autoimmune diseases, hospital admissions secondary to infectious diseases, institutionalized persons, and those with disabilities.	ES 2011
Vitamin D: supplement use in specific population groups. National Institute for Health and Clinical Excellence. 2014 (Updated 2017) [37]	Deficiency 25-(OH)D: <25 nmol/L (<10 ng/mL).	Not provided	25-(OH)D must be only measured when there are symptoms or very high risk of deficiency.	SACN 2016
Recommended vitamin D levels in the general population. Grupo de Trabajo de Osteoporosis y Metabolismo Mineral de la Sociedad Española de Endocrinología y Nutrición, 2017 [38]	They suggest maintaining serum 25-(OH)D concentrations between 75 and 125 nmol/L (30 – 50 ng/mL) to achieve the health benefits of vitamin D. Elevated 25-(OH)D values >125-150 nmol/L (> 50 – 60 ng/mL) could be associated with risk for cardiovascular death or any other cause of death.	Not provided.	Screening only in individuals with risk factors: bones disorders, chronic kidney/hepatic diseases, malabsorption syndromes, hyperparathyroidism, specific medication, pregnant and lactating women, institutionalized persons, obesity, reduced sun exposure, granulomatous disorders, some lymphomas.	ES 2011

Vitamin D in European children-statement from the European Academy of Paediatrics (EAP), 2017 [39]	Sufficiency 25-(OH)D: >50 nmol/L (20 ng/mL). Deficiency 25-(OH)D: <25 nmol/L (10 ng/mL).	Considerable variability exists among the various assays available and among laboratories.	There is no evidence for routine vitamin D screening in healthy children. Testing in situations at risk for deficiency: bones diseases, darker pigmented skin, reduced sun exposure, chronic liver/kidney disease or with malabsorption, dietary inadequacy, obesity, long-term parenteral nutrition, institutionalized children, and with anticonvulsant medication.	IOM 2011
Assessment criteria for vitamin D deficiency/ insufficiency in Japan: proposal by an expert panel supported by the Research Program of Intractable Diseases, Ministry of Health, Labour and Welfare, Japan, the Japanese Society for Bone and Mineral Research and the Japan Endocrine Society, 2017 [40]	Sufficiency 25-(OH) D: ≥ 75 nmol/L (≥ 30 ng/ mL). Insufficiency 25-(OH)D: 50-75 nmol/L (20-30 ng/mL). Deficiency 25-(OH) D: < 50 nmol/L (< 20 ng/mL).	Serum 25-(OH)D level may vary depending on the assay used. Standardization of the assay will be needed.	Not provided	ES 2011
Vitamin D and bone health: A practical clinical guideline for patient management, Royal Osteoporosis Society, 2018 [41]	Deficiency: plasma 25-(OH) D < 25 nmol/L (<10 ng/ mL). Inadequate in some people: plasma 25-(OH)D of 25-50 nmol/L (10 -20 ng/mL) Sufficiency: plasma 25-(OH)D > 50 nmol/L (> 20 ng/mL)	Measurement of plasma 25-(OH)D is the best way for estimating vitamin D status. The assay should have the ability to recognise all forms of 25-(OH)D (D2 or D3) equally. This means that it should use either HPLC-MS/MS. None of the immunoassays offers the ability to recognize all forms of 25-(OH)D.	Universal screening of asymptomatic population is not recommended. They only suggest testing 25-(OH) in patients with musculoskeletal symptoms attributed to vitamin D deficiency, and in situations where malabsorption or poor compliance with medication is suspected.	IOM 2011 SACN 2016

Italian Association of Clinical Endocrinologists (AME) and Italian Chapter of the American Association of Clinical Endocrinologists (AACE) Position Statement: Clinical Management of Vitamin D Deficiency in Adults, 2018 [42]	25-(OH)D concentrations of 50 nmol/L (20 ng/mL) are appropriate in the general population. They recommend maintaining levels above 75 nmol/L (> 30 ng/mL) in situations of risk.	The same method must be used for serial measurement of 25-(OH)D in any patient. The standardization of 25-(OH)D levels by immunoassay methods to LC-MS/MS will provide valid conclusions about the actual health implications of vitamin D deficiency.	Screening of 25-(OH) D is not indicated in healthy people. Testing only 25-(OH) D in: bones disorders, older adults with falls and/or non-traumatic fractures, chronic kidney/ hepatic diseases, cystic fibrosis, malabsorption syndromes, hyperparathyroidism, specific medication, pregnant and lactating women, institutionalized persons, obesity, reduced sun exposure, granulomatous disorders, some lymphomas.	IOM 2011 ES 2011
Vitamin D in pediatric age: consensus of the Italian Pediatric Society and the Italian Society of Preventive and Social Pediatrics, jointly with the Italian Federation of Pediatricians, 2018 [43]	Severe deficiency 25-(OH) D: < 25 nmol/l (< 10 ng/ mL). Deficiency 25-(OH)D: < 50 nmol/L (< 20 ng/ mL). Insufficiency 25-(OH)D: 50-74 nmol/L (20-29 ng/ mL). Sufficiency 25-(OH) D: > 75 nmol/L (> 30 ng/mL). Hypovitaminosis D: < 75 nmol/L (< 30 ng/mL).	Some methods available for determining 25-(OH)D still present poor accuracy and precision. The isotope dilution- LC-MS/MS is considered the best method for measuring serum 25-(OH)D.	Screening 25-(OH)D in healthy individuals is not recommended. 25-(OH) D evaluation should be limited in children and adolescent with risk factors for vitamin D deficiency, in subjects that require supplementation during the whole year or receiving drugs affecting vitamin D metabolism, dark skin, reduced sunlight exposure, obesity, inadequate diets, chronic kidney/hepatic diseases, malabsorption syndromes, chronic therapies.	ES 2011

Recomendaciones para la valoración bioquímica del estatus de Vitamina D. Comisión de Hormonas de la SEQC-ML, 2019 [44]	25-(OH)D concentrations below 25 nmol/L (12 ng/mL) are inadequate, because they are associated with an important increase in the risk for rickets in children and osteomalacia in adults. 25-(OH)D concentrations around 75 nmol/L (30 ng/mL) are adequate for a good bone health. 25-(OH)D concentrations less than 50 nmol/L (20 ng/mL) are suboptimal.	There is lack of agreement of results with the different methods. Most clinical laboratories use automated immunoassays with CDC Certified Vitamin D Program (VDSP), which show acceptable overall correlation with LC-MS/MS methods used as reference. The external quality program DEQAS has shown a gradual reduction in the CV between laboratories.	Screening without risk factors for 25-(OH) D deficiency is not recommended. Patients that should be screened: bones disorders, chronic kidney/hepatic diseases, malabsorption syndromes, specific medication hyperparathyroidism, abnormalities of calcium and/or phosphate metabolism, unexplained high levels of alkaline phosphatase, suspected toxicity. Basal 25-(OH) D level is not necessary in case of: obesity, dark skin, reduced sunlight exposure, institutionalized persons.	IOM 2011 ES 2011
Recomendaciones de la SEIOMM en la prevención y tratamiento del déficit de vitamina D, 2021 [45]	Serum 25-(OH)D levels between 62.5-125 nmol/L (25 - 50 ng/mL) are recommended to achieve the bone health benefits. In patients with osteoporosis or at risk for fracture, 25-(OH)D between 75 – 125 nmol/L (30 - 50 ng/mL) are recommended. Maximum concentration 25-(OH) D: 125 - 220 nmol/L (50-88 ng/mL).	It is recommended that the laboratory have a quality assurance program certification and the standardization of serum 25-(OH)D determinations to minimize analytical variability.	Screening for 25-(OH) D deficiency in people with risk factors: people with weakness muscle and/or risk of falls, dark skin, reduced sunlight exposure, bone diseases, advanced age and/or institutionalized persons, cognitive deficiency, smoking, obesity, inadequate diets, risk of malnutrition, malabsorption syndromes, renal or hepatic insufficiency, hypo and hyperparathyroidism, bones diseases, pregnant and lacting, medications that interfere with cytochrome P450.	ES 2011
Screening for Vitamin D Deficiency in Adults: US Preventive Services Task Force Recommendation Statement, 2021 [46]	More research is needed to determine the cut-off point that defines vitamin D deficiency and whether that limit varies depending on the patient clinical outcome or by subgroups defined by race, ethnicity or sex.	Evidence suggests that results depend on the testing method and vary among laboratories using the same testing methods.	The current evidence on the benefits of screening for vitamin D deficiency is lacking. Therefore, the balance of benefits and harms of screening for vitamin D deficiency in asymptomatic adults cannot be determined.	None

Recommendations on the measurement and the clinical use of vitamin D metabolites and vitamin D bin ding protein – A position paper from the IFCC Committee on bone metabolism, 2021 [47]	Differences exist in the definition of vitamin D deficiency, insufficiency, and sufficiency, creating a great deal of controversy. The most critical factor that confounds efforts to develop consensus in clinical and nutritional public health guidelines for interpreting serum 25-(OH)D concentrations is the substantial variability that still exists in many assays that have been used over time to measure 25-(OH)D. The lack of assay standardization is the main source of bias.	The best sample to measure 25-(OH)D is serum. Many immunoassays suffer from dependent deviations and manufacturers should improve these assays. Standardized LC-MS/MS methods are currently the only tools able to measure 25-(OH)D regardless of the nature of the sample. CDC started an international Vitamin D standardization certification program, led to an improvement in the number of standardized 25-(OH)D assays. Limits for total CV and mean bias should be ≤ 10% and ≤ 5%, respectively, for routine clinical laboratories.	Not provided	None
Recomendaciones de uso adecuado de pruebas y suplementos de Vitamina D en población general. Ministerio de Sanidad, 2021 [48]	There is lack of consensus on optimal 25-(OH)D values, but there is a minimum agreement: >50 nmol/L (>20 ng/mL) is recommended and <25 nmol/L (<10 ng/mL) must be avoided at all ages. Consensus results of expert groups (delphi model) are: Deficiency 25-(OH)D: < 50 nmol/L (< 20 ng/mL). Insufficiency 25-(OH)D: 50-74.75 nmol/L (20-29.9 ng/mL). Optimal 25-(OH)D: 75-125 nmol/L (30-50 ng/mL).	There are different quantification methods available. LC-MS/MS is the gold standard technique.	In asymptomatic healthy adults without risk factors for 25-(OH)D deficiency, there is no proved evidence to test 25-(OH) D levels. Screening is recommended in people with risk factors: bone metabolism alterations, obesity, malabsorption syndromes, and others.	ES 2011
The clinician's guide to prevention and treatment of osteoporosis, 2022 [49]	The current normal range for 25-(OH)D levels is between 75 and 125 nmol/L (30-50 ng/mL). In healthy individuals, serum 25-(OH)D ≥50 nmol/L (≥20 ng/mL) may be sufficient, but in the setting of known or suspected metabolic bone disease ≥75 nmol/L (≥30 ng/mL) is appropriate.	Not provided	Not povided	IOM 2011 ES 2011

Role of vitamin D supplementation in the management of musculoskeletal diseases: update from an European Society of Clinical and Economical Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO) working group, 2022 [50]	Deficiency or severe deficiency 25-(OH)D: < 25nmol/L or <30 nmol/L (<10 ng/mL or <12 ng/mL) (depending on the expert society), when the focus was the prevention of rickets/osteomalacia. Insufficiency or deficiency 25-(OH)D: < 50 nmol/L (<20 ng/mL), if the concern was suppression of PTH.	There is an absolute need for a standardized method. LC-MS/ MS methods generally perform better than immunoassays, but all LCMS/MS methods are not equivalent. 24,25-(OH)2D and VMR (vitamin D metabolite ratio) are promising tools to evaluate vitamin D deficiency.	25-(OH)D testing is appropriate in bones diseases, hyperparathyroidism, malabsorption syndromes, medications affecting metabolism of vitamin D, chronic kidney disease, hypophosphatemia and hypo/hypercalcemia, pigmented skin, and isolated elevation of alkaline phosphatase.	IOM 2011 ES 2011
Vitamin D. Fact Sheet for Health Professionals. National Institute of Health, 2022 [51]	Vitamin D deficiency: <30 nmol/L (<12 ng/mL). Inadequate for bone and overall health in healthy individuals: 30 to <50 nmol/L (12 to <20 ng/mL). Adequate for bone and overall health in healthy individuals: ≥50 nmol/L (≥20 ng/mL) . 25-(OH) D linked to toxicity: >125 nmol/L (>50 ng/mL).	Assessing vitamin D status by measuring serum 25-(OH)D concentrations is complicated by the considerable variability of the available assays. The international VDSP has developed procedures for standardizing the laboratory measurement of 25-(OH) D to improve clinical and public health practice.	There isn't any national professional organization that recommends population screening for vitamin D deficiency in asymptomatic patients.	IOM 2011
Definition, Assessment, and Management of Vitamin D Inadequacy: Suggestions, Recommendations, and Warnings from the Italian Society for Osteoporosis, Mineral Metabolism and Bone Diseases (SIOMMMS), 2022 [52]	<u>In the general population:</u> Deficiency 25-(OH) D: <25 nmol/L (<10 ng/ mL). Insufficiency 25-(OH)D: < 50 nmol/L (20 ng/ mL). Optimal 25-(OH)D: 50-124.8 nmol/L (20–50 ng/mL). <u>Population at risk or treatment with bone modifying agents:</u> Deficiency 25-(OH)D: <25 nmol/L (<10 ng/ mL). Insufficiency 25-(OH)D: < 74.9 nmol/L (< 30 ng/mL). Optimal 25-(OH)D: 74.9-124.8 nmol/L (30–50 ng/mL).	There is an urgent need for standardization/ harmonization for a correct interpretation of clinical studies and for clinical practice. The assessment of serum 25-(OH)D levels is mostly performed using immunochemiluminescence methods with intra-assay and inter-assay variability of 10-20%. The LC-MS/ MS is considered the most accurate and precise method for research and clinical use.	It is recommended not to perform 25-(OH) D measurement in the general population. Measurement of 25-(OH)D levels is only recommended when it is necessary for the clinical management of the patient.	IOM 2011 ES 2011
Vitamin D – a scoping review for Nordic nutrition recommendations 2023 [53]	There is a growing agreement that : Deficiency 25-(OH)D: <25-30 nmol/L (<10-12 ng/mL). Sufficiency 25-(OH)D: > 50 nmol/L (>20 ng/ mL).	All measurements should be standardized. The LC-MS/MS is considered the most valid method for measurement of Vitamin D metabolites.	Not provided	IOM 2011

Guidelines for preventing and treating vitamin D deficiency: A 2023 Update in Poland, 2023 [54]	Deficiency 25-(OH) D: < 50 nmol/L (< 20 ng/mL). Insufficiency 25-(OH)D: 50-75 nmol/L (20-30 ng/mL). Sufficiency 25-(OH) D: 75-125 nmol/L (30-50 ng/mL). Toxicity 25-(OH)D: > 250 nmol/L (> 100 ng/mL).	The measure of 25-(OH)D should be subject to quality assurance by the certifying system DEQAS.	The screening of serum 25-(OH)D is not recommended. In the risk group is strongly recommended: increased demand for physiological reasons, malabsorption syndromes, diseases of liver and bile ducts, respiratory diseases, infectious diseases, systemic connective tissue diseases, skin diseases, diseases of nervous system, decreases production of vitamin D3 in the skin, nutritional features, long-term use of drugs, malignant neoplasms, granulomatous diseases, mental illness, cardiovascular diseases, chronic fatigue syndrome, inpatient treatment, pre and post-transplant.	ES 2011

25-(OH)D: 25-hydroxyvitamin D; 25-(OH)D2: 25-hydroxyvitamin D2; 25-(OH)D3: 25-hydroxyvitamin D3; 24,25-(OH)2D: 24,25-dihydroxyvitamin D; AIDS: acquired immunodeficiency syndrome; CDC: Centers for Disease Control and Prevention; CV: coefficient of variation; DEQAS: vitamin D external quality program assessment scheme; ES: Endocrine Society; IOF: International Osteoporosis Foundation; IOM: Institute of Medicine; HPLC: high pressure liquid chromatography; LC-MS/MS: liquid chromatography-tandem mass spectrometry; NIST: The National Institute of Standards and Technology; PTH: parathyroid hormone; SACN: Scientific Advisory Committee on Nutrition; VDSP: Vitamin D Standardization Program.

There is uniform consensus about the lack of need for general population screening [46]. However, there are differences regarding the target population for vitamin D deficiency screening. There is also an agreement on the need for using standardized methods to measure 25-(OH)D as an indicator of vitamin D status, and on the participation in external quality programs established by the Vitamin D Standardization Program (VDSP).

The main controversial aspects of vitamin D related to clinical laboratory from the three international conferences held among 2017 and 2019 are displayed in Table 3 [13-15]. These conferences highlight the need for standardization of the methodology to determine 25-(OH)D.

Table 3: Laboratory aspects at International Conferences on controversies in vitamin D between 2017 – 2019.

Representative articles from International Conferences (2017-2019) on controversies in vitamin D	**Summary of laboratory aspects**
Controversies in vitamin D: Summary Statement from an International Conference (Pisa, June 2017) [13]	Available guidelines suggest that 25-(OH)D values <12 ng/mL (< 30 nmol/L) are associated with an increased risk of rickets/osteomalacia, whereas 25-(OH)D concentrations between 20 and 50 ng/mL (50 to 125 nmol/L) appear to be safe and sufficient for skeletal health in the healthy general population. It is not clear whether these guidelines should be considered with regards to individuals who have metabolic bone diseases, such as osteoporosis or primary hyperparathyroidism. Need for a standardized determination of 25-(OH)D concentration is crucial for a clearer definition of vitamin D status: deficiency, sufficiency or excess.
Consensus Statement from 2nd International Conference on Controversies in Vitamin D (Siena, September 2018) [14]	Existing data are insufficient to define with certainty low or high vitamin D status thresholds because of the lack of standardized 25-(OH)D measurements. Defining vitamin D status using serum 25-(OH)D concentration with standardized methodology is recommended. Assays should demonstrate standardization or alignment with reference methodology proposed by the VDSP. Laboratories should participate in a 25-(OH)D accuracy program (DEQAS or CAP). Manufacturers should develop assays with ability to accurately measure 25-(OH)D2 and 25-(OH)D3 in various clinical circumstances. The risk for developing rickets/osteomalacia is increased at a 25-(OH)D concentration ≤ 12 ng/mL (30 nmol/L). This threshold may vary depending on other conditions such as calcium and phosphate nutrition, parathyroid hormone (PTH) levels, and season. The 25-(OH)D concentration ranges among normal subjects are between 50 and 125 nmol/L. An upper 25-(OH)D threshold of 125 nmol/L is advisable.
Controversies in Vitamin D: A Statement from the Third International Conference (Gubbio, September 2019) [15]	Severe vitamin D deficiency, defined as <12 ng/mL (30 nmol/L) is seen in approximately 7% of the population worldwide, with variation among countries and populations. The circulating 25-(OH)D concentration is widely accepted as the best marker of vitamin D status, although with little physiologic regulation. There is ongoing debate with regard to whether free 25-(OH)D or the ratio [24,25-(OH)2D]/[25-(OH)D] is a superior marker than total 25-(OH)D. There is consensus that 25-(OH)D levels below 12 ng/mL (30 nmol/L) are clearly deficient and levels above 30 ng/mL (75 nmol/L) are clearly sufficient. There is disagreement on levels between 12 and 30 ng/mL (30 and 75 nmol/L). Some guidelines recommend a threshold value of 20 ng/mL (50 nmol/L), whereas others aim for ≥30 ng/mL (≥ 75 nmol/L). This discussion is largely based on the lack of 25-(OH)D assay standardization.

25-(OH)D: 25-hydroxyvitamin D; 25-(OH)D2: 25-hydroxyvitamin D2; 25-(OH)D3: 25-hydroxyvitamin D3; 24,25-(OH)2D: 24,25-dihydroxyvitamin D; CAP: college of American pathologists; DEQAS: vitamin D external quality program assessment scheme; VDSP: Vitamin D Standardization Program.

Discussion

This review identified and scrutinized, from data of the main guidelines, three major issues related to vitamin D status assessment: the difficulty in defining the desirable levels, which may vary according to underlying conditions, the variability in the assay methodology, and the need of standardization. Indeed, these controversial topics were also considered as major issues in a recent study [55].

Despite global consensus on the need to use standardized methodology to correctly determine vitamin D status in the general population, guidelines and/or recommendations continue to take into consideration studies from the IOM, the ES, or both, when at the time of their publication there was not a standardized methodology.

Another remarkable controversy is the origin of the ES recommendations, based on the IOF recommendations derived from randomized clinical trials in adult population [12] and being a guide for patients with chronic disorders, as clarified one year later by the same working group of the ES [56]. In our opinion, a methodological and population bias appears in the guidelines and recommendations that only take into consideration one of the possible indications: the IOM recommendations are aimed at the general population, while the Endocrine Society guideline is based on the needs of population with chronic pathologies that can affect bone metabolism. Given the different goals of the IOM and the ES clinical practice guideline, it is not surprising that their recommendations differed. This situation, together with the rise of publications with contradictory results from the majority of observational studies, is producing a lack of agreement between clinical laboratories to establish recommendations to measure 25-(OH)D and reference intervals to establish vitamin D status depending on the type of population.

Regarding methodological aspects, clinical laboratories must be aware of their analytical limitations for the correct interpretation of results. Due to the increasing number of samples received by routine clinical laboratories, the use of an automated methodology and, therefore, immunoassays certified by the Center of Disease Control and Prevention (CDC) for vitamin D are necessary [57].

The latest published results from DEQAS [17] indicate that, although the results from immunoassays have reduced the imprecision among methods, a bias continues to appear in low and high values, and non-assessment of the 25-(OH)D2 metabolite may not reflect vitamin D status when supplementation is performed with vitamin D2. To understand these limitations, it is important to participate in an external quality program that meets the VDSP criteria.

After the review of the existing evidence, the current situation would be as follows: it is generally accepted that 25-(OH)D concentrations < 25 nmol/L (<12 ng/mL) are deficient and can affect bone and musculoskeletal health, and that concentrations > 75 nmol/L (> 30 ng/mL) are sufficient for any type of population (age, ethnic group and pathophysiological condition, with or without risk for vitamin D deficiency). The controversy appears in concentrations between 25-75 nmol/l (12-30 ng/mL), in wich the definition of vitamin D status will depend on age and risk factors. This way, concentrations between 25-50 nmol/L (12-20 ng/mL) may be sufficient for some people, but not for the entire healthy population. Therefore, and in accordance with the recommendations of the IOM [10] and the ES [11], concentrations > 50 nmol/L (> 20 ng/mL) are sufficient for a healthy population without risk factors under 60-65 years, and concentrations > 75 nmol/L (> 30 ng/mL) are sufficient for the global population, and necessary in patients with risk factors, regardless of age.

Another important item reviewed is when the determination of the concentration of 25-(OH)D is indicated. There is agreement about not performing screening in population without risk of vitamin D deficiency, being reinforced with the publication on 2021 of the US Preventive Services Task Force, in the latest consensus on vitamin D resulting from the 6th International Conference on Vitamin D and in the recent guideline published by the ES [46, 55, 58]. There is also consensus in measuring 25-(OH)D in symptomatic patients and in those at risk of deficiency. However, there is no accordance in defining risk situations of vitamin D deficiency that do require such determination. The most recent guidelines and recommendations agree on analyzing population with bone disorders (osteoporosis, rickets, osteomalacia, unjustified fractures, alterations in phosphocalcium metabolism, hypo- and hyperparathyroidism, elevated alkaline phosphatase without justification), chronic kidney and liver diseases, malabsorption and medication that interferes at the cytochrome P450 levels, as it is described in Table 2. There is also a recommendation to directly supplement without measuring levels in patients at risk of suffering from deficiency, but without chronic diseases: little sun exposure, institutionalized people, or dark-skinned and obese people [19,31,41,44, 58].

The assessment of vitamin D status becomes relevant especially when the refundability of vitamin D supplements depends on governmental criteria, sometimes diverging from guidelines due to lack of consensus [4]. For this reason, clinical laboratories must make an effort and unify reports to facilitate clinical decision-making: it would be convenient to use the units of the international system of nomenclature (nmol/L), to report not reference range but clinical decision values, and it is crucial for all laboratories to be aware of the performance and limitations of their 25-(OH)D assays to ensure the reliable assessment of vitamin D status.

In conclusion, although there have been advances in methodology, with automatized methods and traceable calibrators by the CDC standards, there is a paralysis in the development of current population studies with standardized methodology to accurately establish the status of vitamin D in both healthy population and population at risk for vitamin D deficiency.

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Research Article

Correlation Analysis of Direct LDL Measurement and Calculated LDL Methods in Lipid Profile Assessment: A Comprehensive Study

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Keywords

LDL-cholesterol, Freidewald equation, various modified formulae, Vujovic

Abstract

Introduction

Assessing LDL cholesterol is pivotal for cardiovascular risk evaluation. While direct LDL measurement is accurate, calculated LDL methods offer practicality and cost-effectiveness. This study aims to evaluate the correlation between direct LDL measurement and various calculated LDL methods, shedding light on their clinical utility.

Methods

A retrospective analysis of lipid profiles from 1075 patients was conducted, encompassing direct LDL measurement and calculation of LDL using nine different methods. Statistical analyses, including correlation coefficients and scatter plots, were employed to assess the agreement between direct LDL and calculated LDL methods.

Results

Surprisingly, all calculated LDL methods exhibited a robust correlation with direct LDL measurement across the study cohort. The Friedewald equation, as well as modified equations demonstrated particularly robust correlations. These findings indicate the reliability of calculated LDL methods in estimating LDL cholesterol levels.

Discussion

The significant correlation observed between direct LDL measurement and calculated LDL methods underscores the clinical utility of the latter. While direct LDL measurement remains the gold standard, calculated LDL methods offer practical advantages, particularly in resource-limited settings.

Conclusion

In conclusion, this study highlights the excellent correlation between direct LDL measurement and calculated LDL methods in lipid profile assessment. Clinicians can leverage calculated LDL methods as reliable alternatives for LDL

cholesterol estimation, facilitating efficient cardiovascular risk evaluation in routine clinical practice. Further research may explore the optimal use of calculated LDL methods in specific patient populations, enhancing their clinical applicability and utility.

Introduction

One among the leading cause of mortality worldwide are cardiovascular diseases. Low density lipoprotein (LDL) are considered bad cholesterol as it causes atherosclerosis, an utmost contributor to cardiovascular disease [1]. Low-density lipoprotein-cholesterol (LDL-C) remains of utmost clinical importance; it is positioned in clinical trials as a treatment target and is emphasized in worldwide guidelines as the primary cholesterol target [2]. It is mainly due to economic reasons, instead of the direct measurement of LDL-C, the calculation methods are widely used in clinical laboratories particularly in developing countries [3]. In addition to Friedewald Formula, there are several other formulas for calculation of LDL-C such as Chen, de Cordova, Vujovic, Anandaraja, Hattori, Ahmadi, Puavillai, Sampson's equation, Martin-Hopkins, Saiedullah; Planella and Wagner which have not been validated in varied populations [4-15].

Friedwald, the most commonly used formula has its own limitations as shown by earlier studies [16,17]. Over and under estimation of LDL-C in patients suffering from diabetes mellitus, alcoholic liver disease, and chronic liver failure have been seen by many [18-21], which may become a problem to patients. This can be overcome by establishing a formula for our population for which we conducted the following study.

9 different formulas as shown in Table 1. were used along with direct LDL measurement

Table 1: 9 different formulas as shown in this table were used along with direct LDL measurement.

Proposed by	Formula
Friedewald et al., [4]	LDL-C = TC – HDL-C – 0.2 × TG
Ahmadi et al., (5)	LDL-C = TC/1.19 + TG/1.9-HDL-C/1.1
Anandaraja et al., [6]	LDL-C = (0.9 × TC) – (0.9 × TG/5) – 28
Chen et al., (7)	LDL-C = (TC – HDL-C) × 0.9 – (TG × 0.1)
Cordova and Cordova [8]	LDL-C =3/4 (TC-HDLc)
Hattori et al., [9]	LDL-C = (0.94 × TC) – (0.94 × HDL-C) –(0.19 × TG)
Puavillai et al., [10]	LDL-C=TC-HDLc-TG/6
Sampson's equation (3)	LDL-C = [TC/0.948−HDL-C/0.971−(TG/8.56+TG × non-HDL-C/2140 −TG^2/16100)−9.44 [25]]
Vujovic et al., [11]	LDL-C=TC-TG/6.85-HDLc

Materials and methods

A retrospective analysis of lipid profiles from 1078 patients was conducted from clinical biochemistry lab database at SMCH, Trichy for 6 months encompassing direct LDL measurement and calculation of LDL using nine different methods. Institutional ethical committee clearance was obtained (IEC No. 18/2022). Care was taken to anonymised the patients except for age & gender.

All patients who came for complete lipid profile investigation were included

A total of 1075 patients out of 1078 were subdivided into various groups for further analyses based on **age, triglyceride (TG), total cholesterol (TC) & HDL- cholesterol (HDL-C) levels** as in Tables 2-5.

Table 2: Four groups based on age (<20, 20–39, 40–59 and ≥ 60 years).

Age	No. (% age)	Mean Age ± SD	Mean TC (mmol/L) ± SD	Mean TG (mmol/L) ± SD	Mean HDL (mmol/L) ± SD	Mean D-LDL (mmol/L) ± SD
< 20	14 (1.3)	13.79 ± 3.53	3.81 ± 1.05	1.33 ± 0.85	1.12 ± 0.22	2.41 ± 1.02
20-39	200 (18.6)	32.02 ± 5.39	4.55 ± 1.18	1.64 ± 0.82	1.15 ± 0.29	3.01 ± 1.02
40-59	541 (50.3)	49.81 ± 5.43	4.70 ± 1.12	1.74 ± 0.78	1.20 ± 0.68	3.12 ± 1.02
>=60	320 (29.8)	67.20 ± 6.38	4.52 ± 1.06	1.58 ± 0.72	1.14 ± 0.28	2.98 ± 0.95

Table 3: Five levels of TG (<0.56, 0.56–1.69, 1.70–3.38, 3.39–4.51 and > 4.51 mmol/L).

TG mmol/L	No. (% age)	Mean Age ± SD	Mean TC (mmol/L) ± SD	Mean TG (mmol/L) ± SD	Mean HDL (mmol/L) ± SD	Mean D-LDL (mmol/L) ± SD
< 0.56	15 (1.4)	42.27 ± 21.22	3.22 ± 0.86	0.48 ± 0.09	1.18 ± 0.28	2.05 ± 0.67
0.56–1.69	630(58.6)	51.41 ± 14.15	4.42 ± 1.06	1.19 ± 0.30	1.22 ± 0.57	2.95 ± 1.00
1.70–3.38	376 (35)	51.53 ± 13.50	4.91 ± 1.08	2.23 ± 0.42	1.11 ± 0.46	3.24 ± 0.96
3.39–4.51	54 (50.2)	48.78 ± 12.89	5.20 ± 1.21	3.77 ± 0.25	1.03 ± 0.27	3.08 ± 1.12
> 4.51	3	Data excluded due to insufficiency				

Table 4: Three levels of TC (<5.17, 5.17–6.18, >6.18 mmol/L).

TC mmol/L	No. (% age)	Mean Age ± SD	Mean TC (mmol/L) ± SD	Mean TG (mmol/L) ± SD	Mean HDL (mmol/L) ± SD	Mean D-LDL (mmol/L) ± SD
< 5.17	750 (69.8)	51.03 ± 14.62	4.06 ± 0.77	1.55 ± 0.72	1.13 ± 0.60	2.63 ± 0.77
5.17–6.18	244 (22.7)	52.08 ± 12.60	5.58 ± 0.30	1.89 ± 0.80	1.24 ± 0.28	3.77 ± 0.63
> 6.18	81 (7.5)	50.02 ± 12.16	6.81 ± 0.63	2.16 ± 0.87	1.34 ± 0.25	4.75 ± 0.82

Table 5: Three levels of HDLC (<1.03, 1.03–1.52, >1.52 mmol/L).

HDL mmol/L	No. (%age)	Mean Age ± SD	Mean TC (mmol/L) ± SD	Mean TG (mmol/L) ± SD	Mean HDL (mmol/L) ± SD	Mean D-LDL (mmol/L)± SD
< 1.03	340 (31.6)	51.24 ± 14.93	4.05 ± 1.06	1.86 ± 0.81	0.86 ± 0.15	2.75 ± 1.00
1.03–1.52	635 (59.1)	51.37 ± 13.74	4.82 ± 1.01	1.62 ± 0.75	1.22 ± 0.13	3.16 ± 0.95
>1.52	100 (9.3)	49.93 ± 12.52	5.21 ± 1.18	1.37 ± 0.65	1.92 ± 1.37	3.32 ± 1.12

Venous blood samples (3ml) of subjects under strict overnight fasting [8-10hrs] was collected under aseptic precautions. After serum separation immediate analysis of serum lipid profile including direct LDL was done.

Statistical analysis

Statistical analyses, including correlation coefficients and scatter plots, were employed to assess the agreement between direct LDL and calculated LDL methods using SPSS Software version 27.0 and Excel sheet

Mean and standard deviation was used to convey the data.

The data was more thoroughly analysed using Pearson's correlation, Bland-Altman plots and paired t-test was also utilise to compare means of different groups.

Pearson's correlation 'r' near to 1 and p <0.05 was taken as significant

Bland-Altman plots (Figure 1) were used to see the agreement or disagreement between two different methods

Two tailed p-value <0.05 was taken as significant

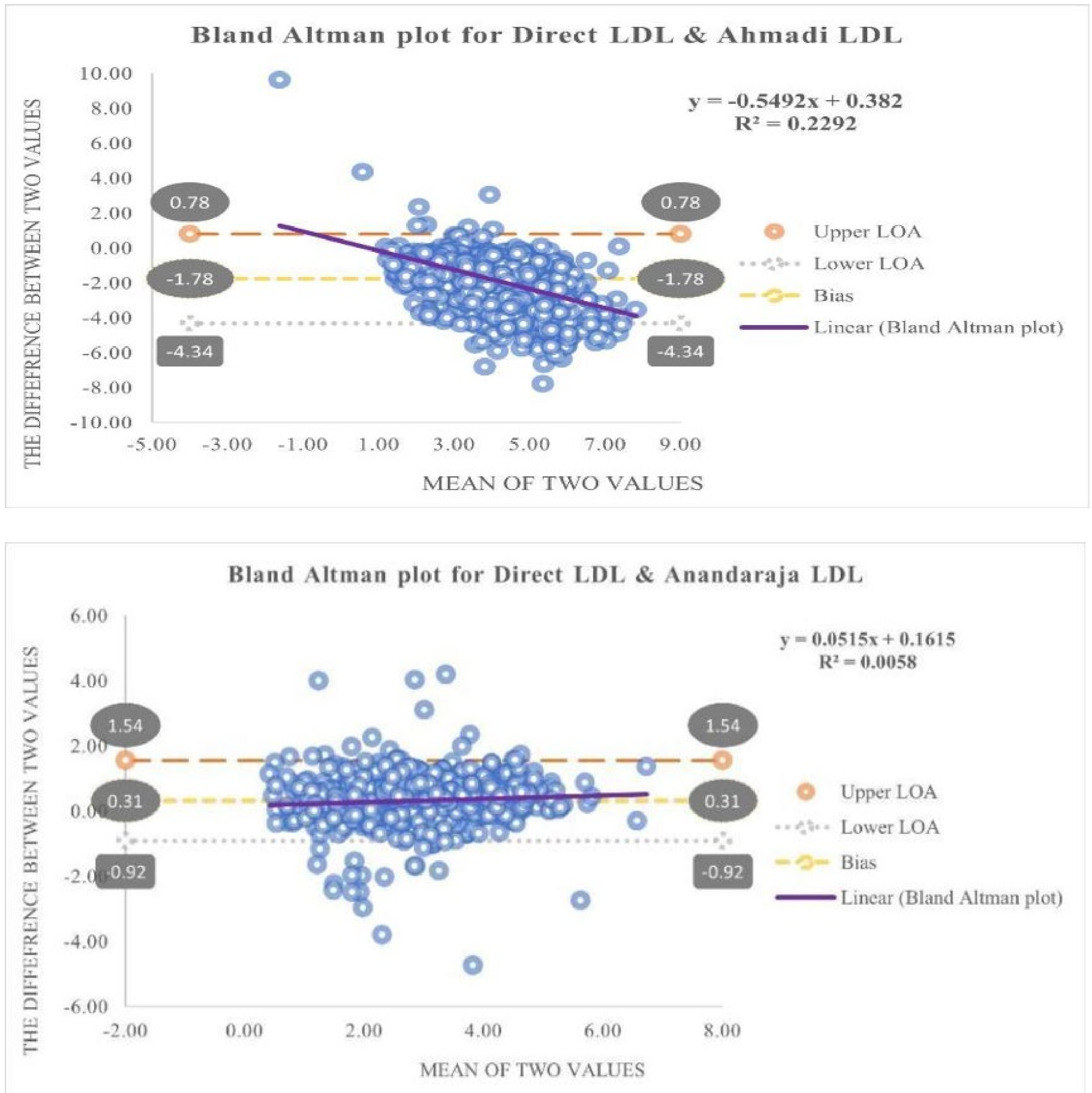
Conversion of TG in mg/dl to mmol/L was done using TG in (mg/dl) /88.57 and for TC, HDL-C and LDL-C values in mg/dl were divided by 38.67

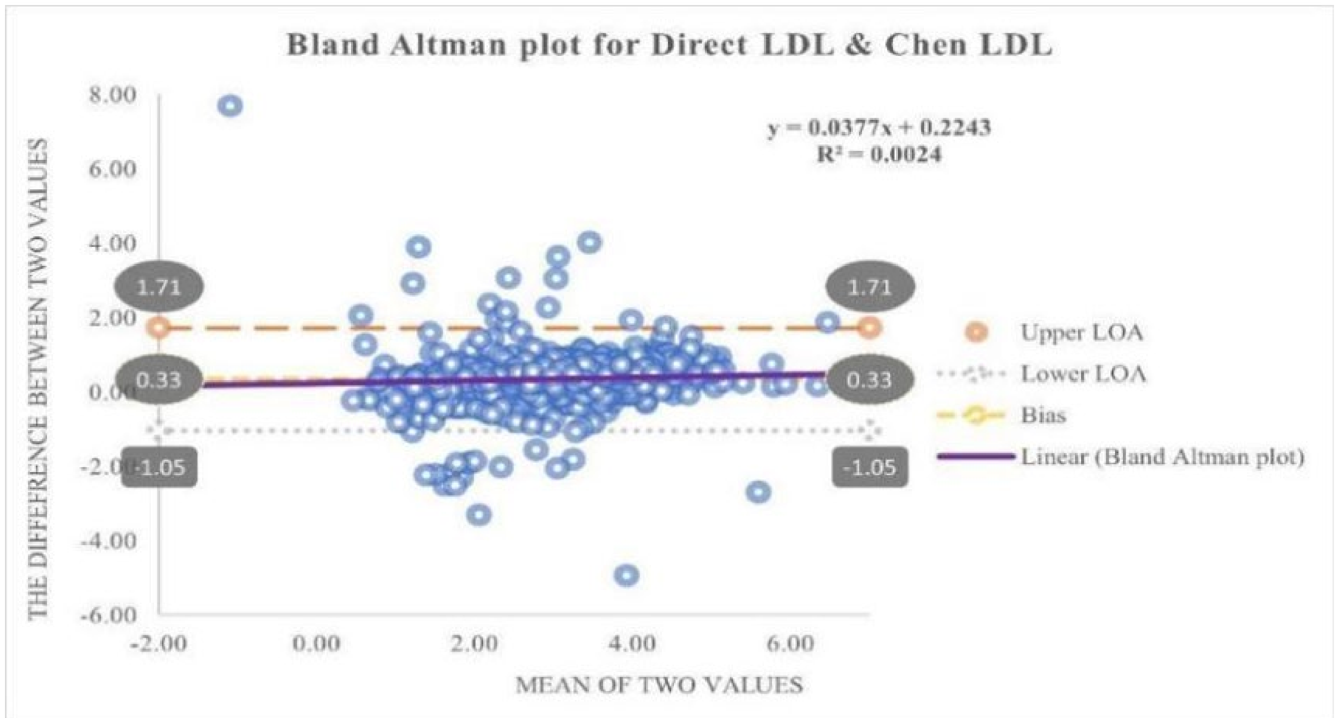
Results

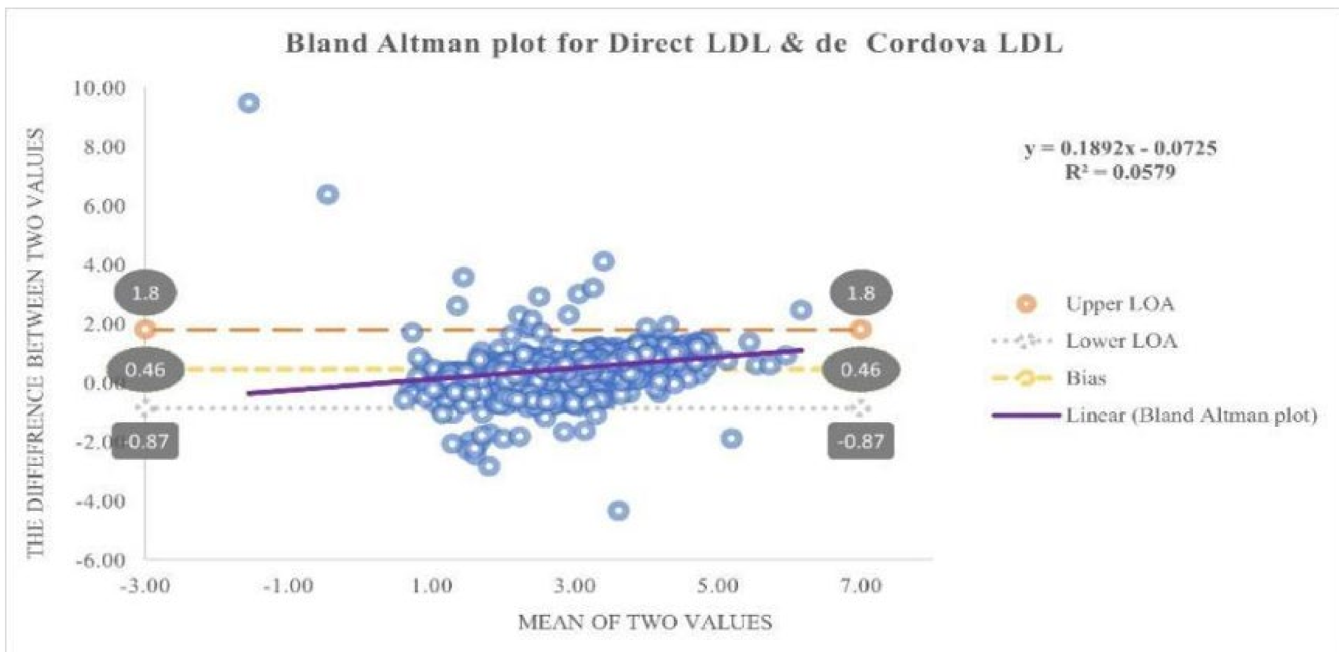
A total of 1075 patients of which 50.5% (543) were females and 49.5% (532) were males with mean age group of 51.19 ± 14.01 years were included. Table 6 shows demographic and lipid data of studied population with mean ± SD, mean difference, p value of paired t-test and r and p of Pearson correlation serving to compare and correlate different formulae

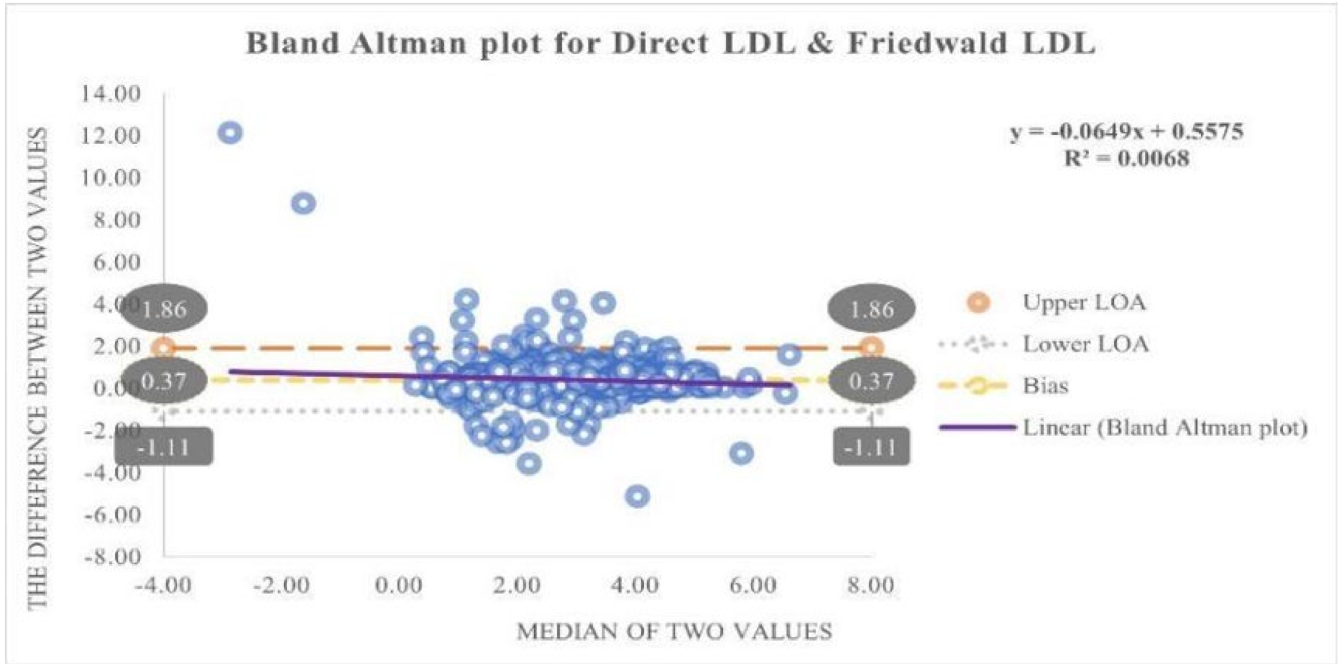
Lowest bias 0.16 is shown by Vujovic formula with lower limit being -1.3 and upper limit being 1.63. The Bland Altmann plot (Figure 1). indicates high level of agreement between Vujovic formula and Direct measurement of LDL. The small bias and narrow limits of agreement suggest that the two methods can be used interchangeably without significant concern for clinical differences. Highest mean difference is shown by Ahmadi formula which means there is small but consistent bias.

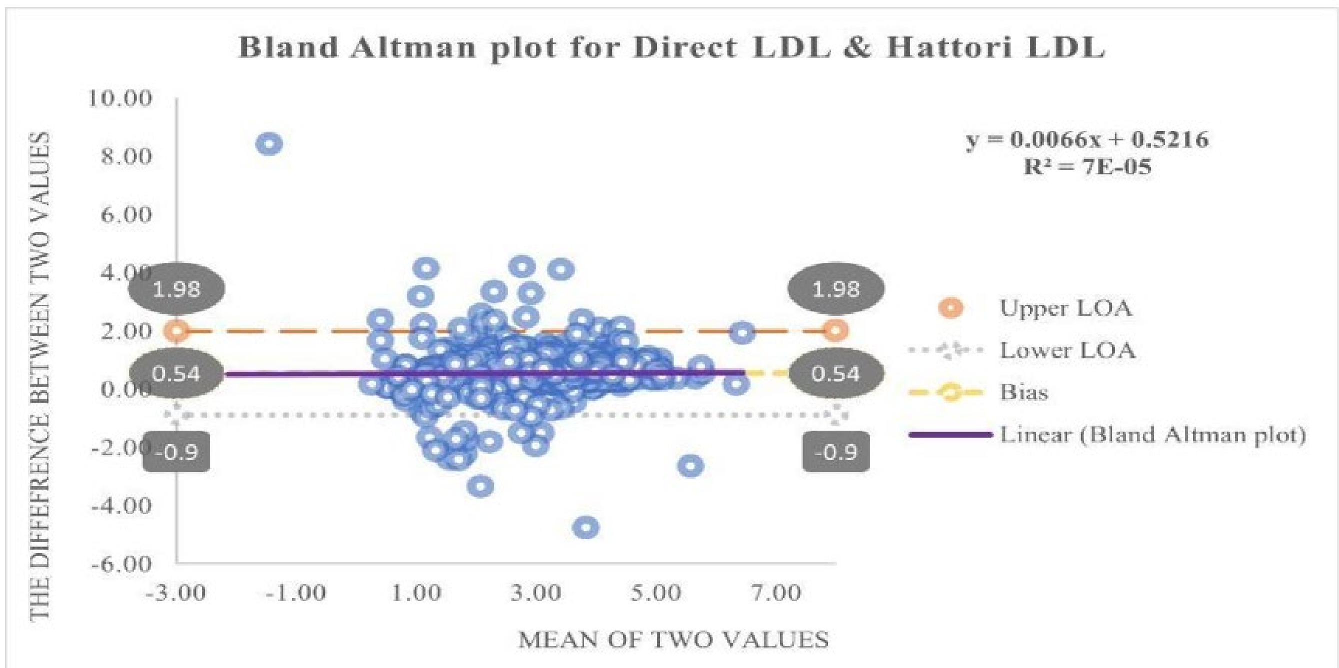
Figure 1: Bland Altman plots to look for bias between Direct-LDL and calculated-LDL's.

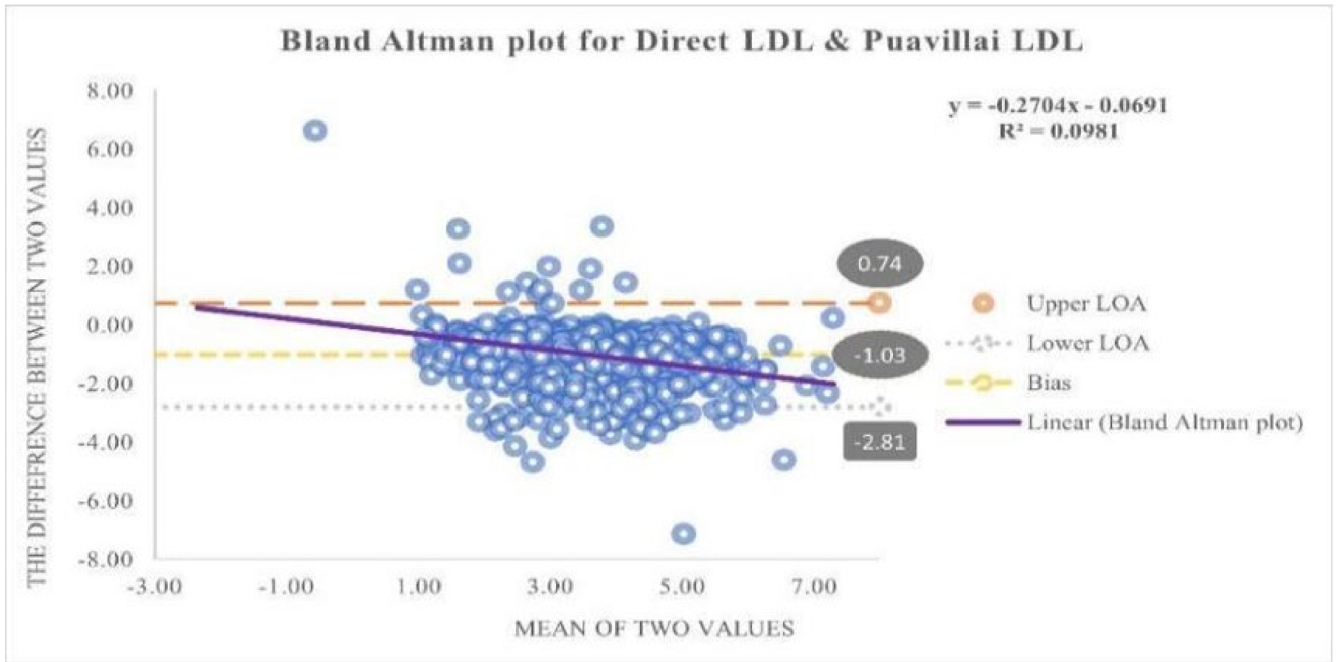


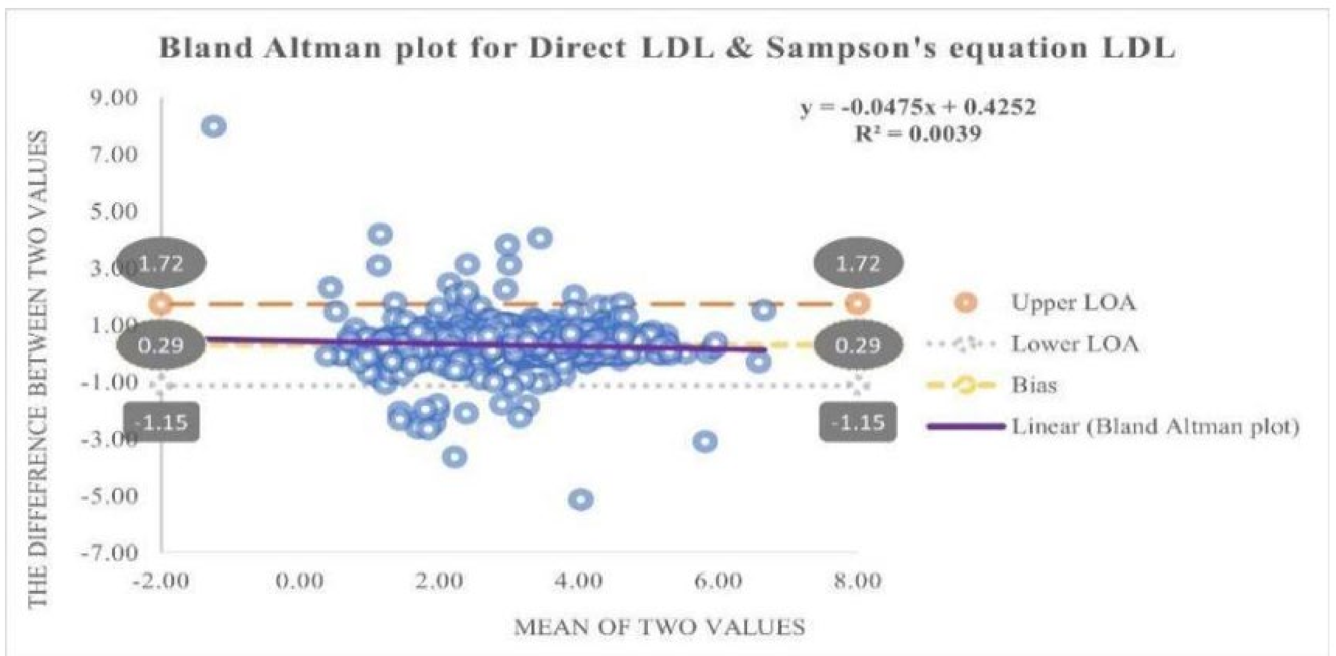

Bland Altman plot for Direct LDL & Chen LDL
y = 0.0377x + 0.2243
R² = 0.0024
1.71
0.33
-1.05
THE DIFFERENCE BETWEEN TWO VALUES
MEAN OF TWO VALUES
Upper LOA
Lower LOA
Bias
Linear (Bland Altman plot)

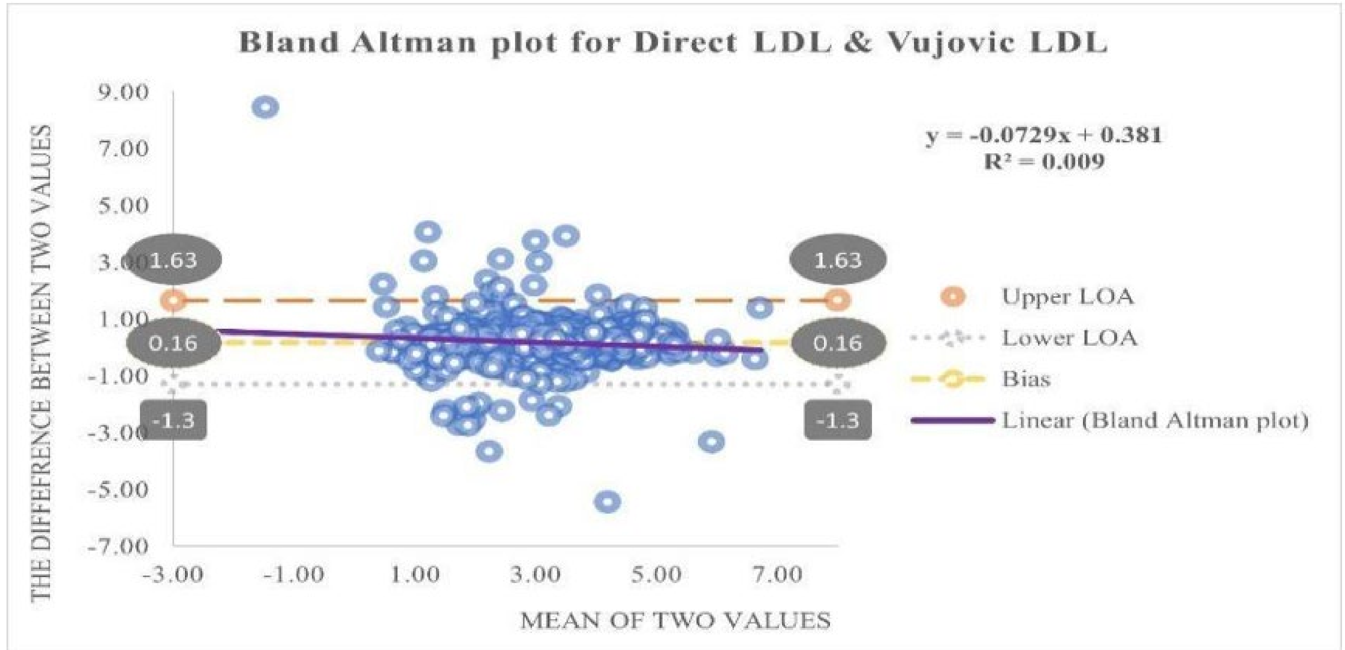

Bland Altman plot for Direct LDL & de Cordova LDL
y = 0.1892x - 0.0725
R² = 0.0579
1.8
0.46
-0.87
THE DIFFERENCE BETWEEN TWO VALUES
MEAN OF TWO VALUES
Upper LOA
Lower LOA
Bias
Linear (Bland Altman plot)


Bland Altman plot for Direct LDL & Friedwald LDL
THE DIFFERENCE BETWEEN TWO VALUES
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
-2.00
-4.00
-6.00
-8.00
-4.00
-2.00
0.00
2.00
4.00
6.00
8.00
MEDIAN OF TWO VALUES
y = -0.0649x + 0.5575
R² = 0.0068
1.86
0.37
-1.11
Upper LOA
Lower LOA
Bias
Linear (Bland Altman plot)


Bland Altman plot for Direct LDL & Hattori LDL
THE DIFFERENCE BETWEEN TWO VALUES
10.00
8.00
6.00
4.00
2.00
0.00
-2.00
-4.00
-6.00
-3.00
-1.00
1.00
3.00
5.00
7.00
MEAN OF TWO VALUES
y = 0.0066x + 0.5216
R² = 7E-05
1.98
0.54
-0.9
Upper LOA
Lower LOA
Bias
Linear (Bland Altman plot)


Bland Altman plot for Direct LDL & Puavillai LDL
y = -0.2704x - 0.0691
R² = 0.0981
THE DIFFERENCE BETWEEN TWO VALUES
8.00
6.00
4.00
2.00
0.00
-2.00
-4.00
-6.00
-8.00
-3.00
-1.00
1.00
3.00
5.00
7.00
MEAN OF TWO VALUES
0.74
-1.03
-2.81
Upper LOA
Lower LOA
Bias
Linear (Bland Altman plot)

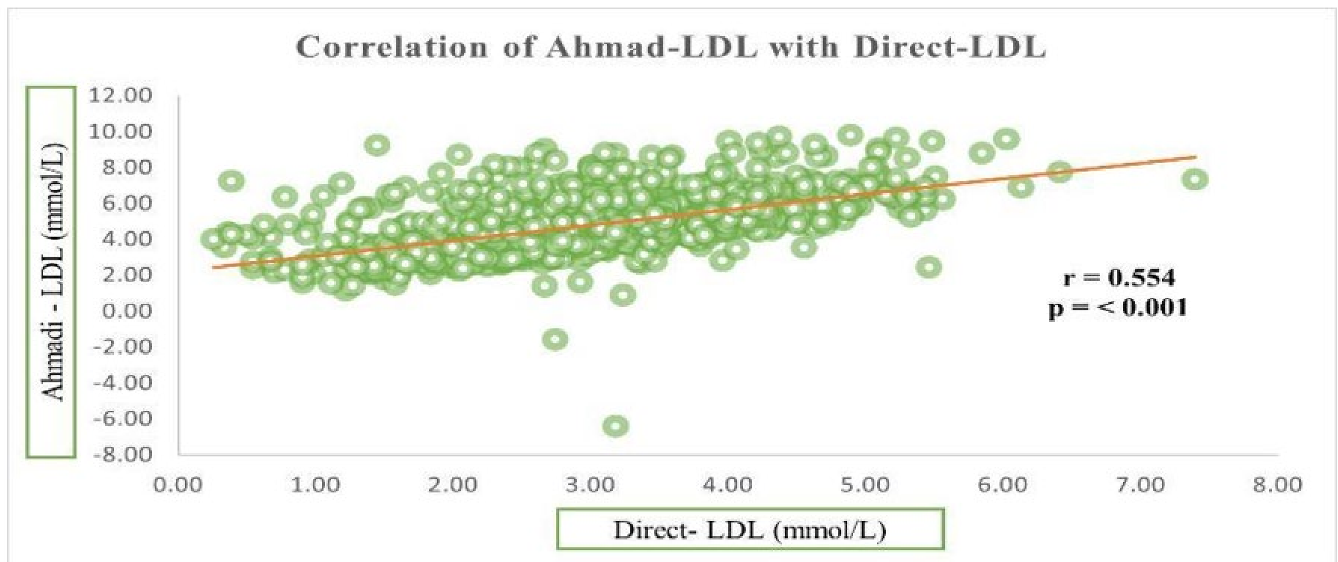

Bland Altman plot for Direct LDL & Sampson's equation LDL
y = -0.0475x + 0.4252
R² = 0.0039
THE DIFFERENCE BETWEEN TWO VALUES
9.00
7.00
5.00
1.72
0.29
-1.00
-1.15
-5.00
-7.00
-2.00
0.00
2.00
4.00
6.00
8.00
MEAN OF TWO VALUES
Upper LOA
Lower LOA
Bias
Linear (Bland Altman plot)

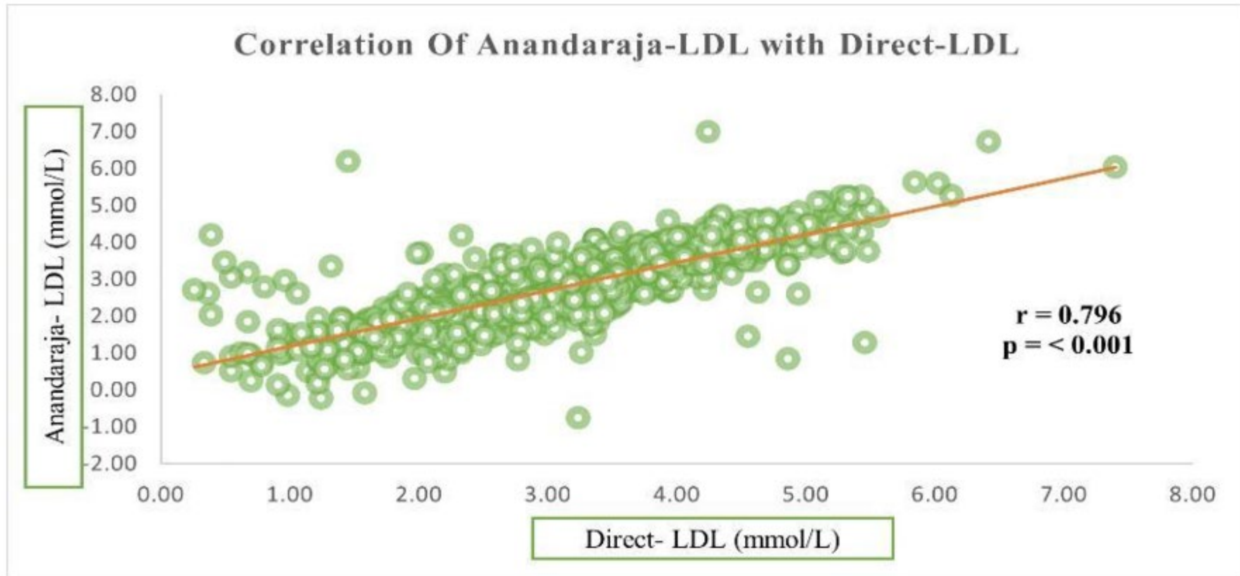


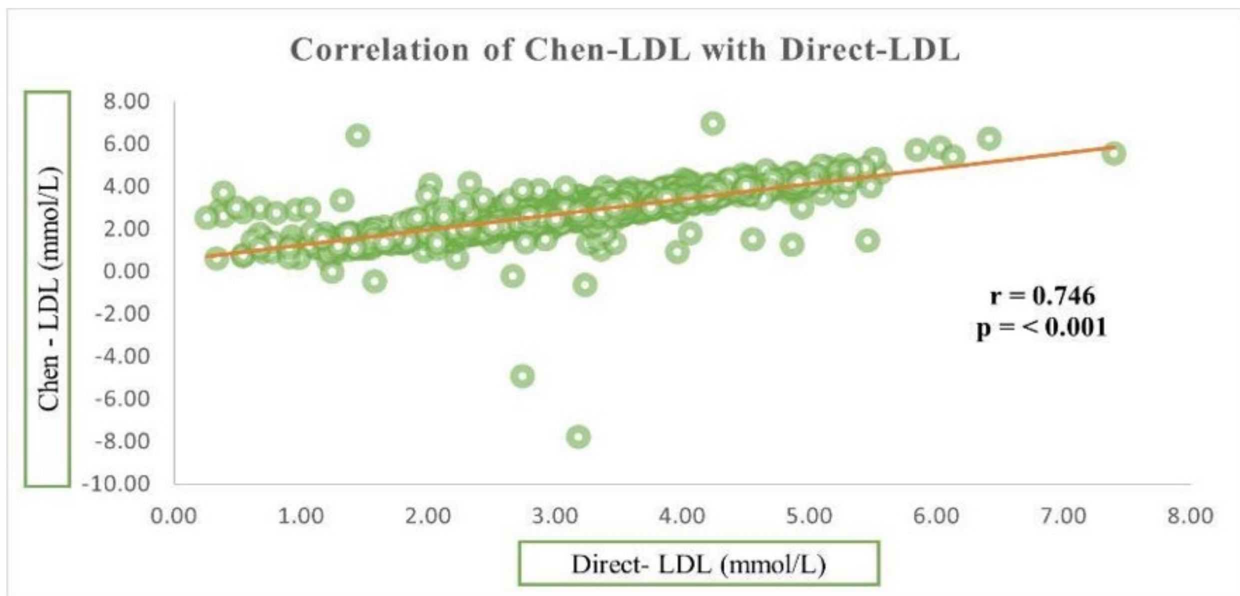
Bland Altmann plots to look for bias and agreement between Direct-LDL and calculated-LDL's

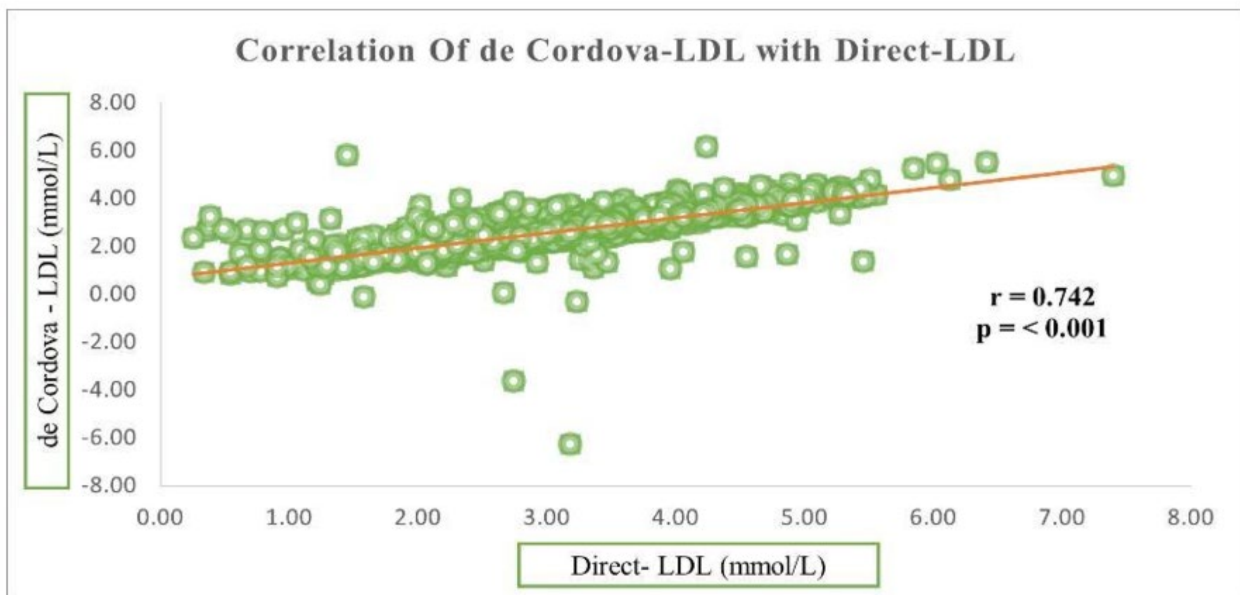
Moderate to strong relation of 0.554 – 0.796 was observed between various calculated formulae with direct LDL (Figure 2).

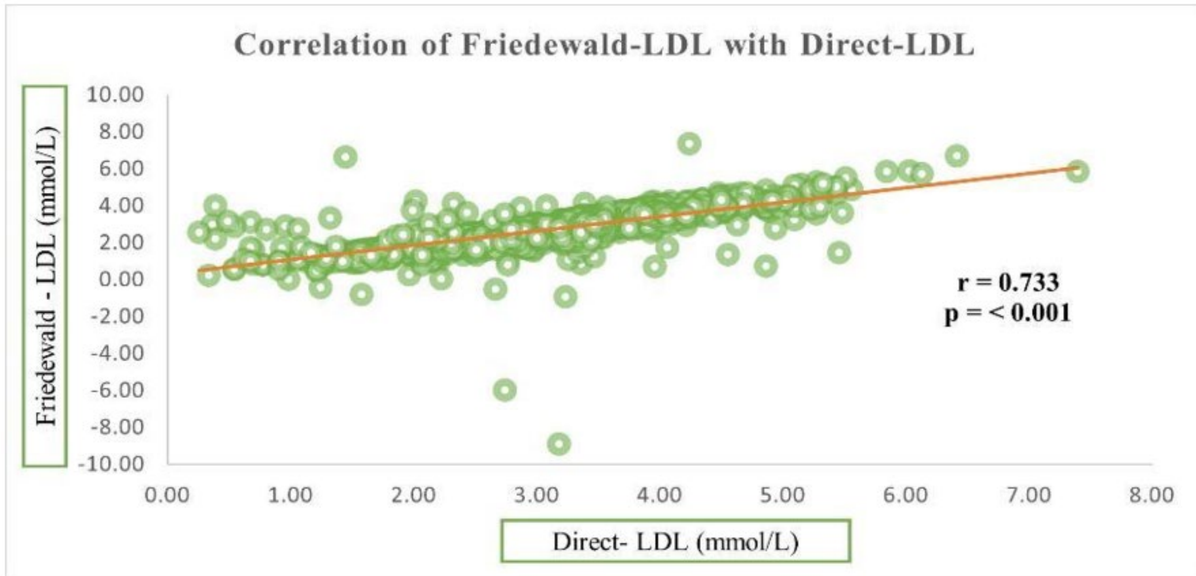
Figure 2: Correlation of various calculated formulae with direct LDL.

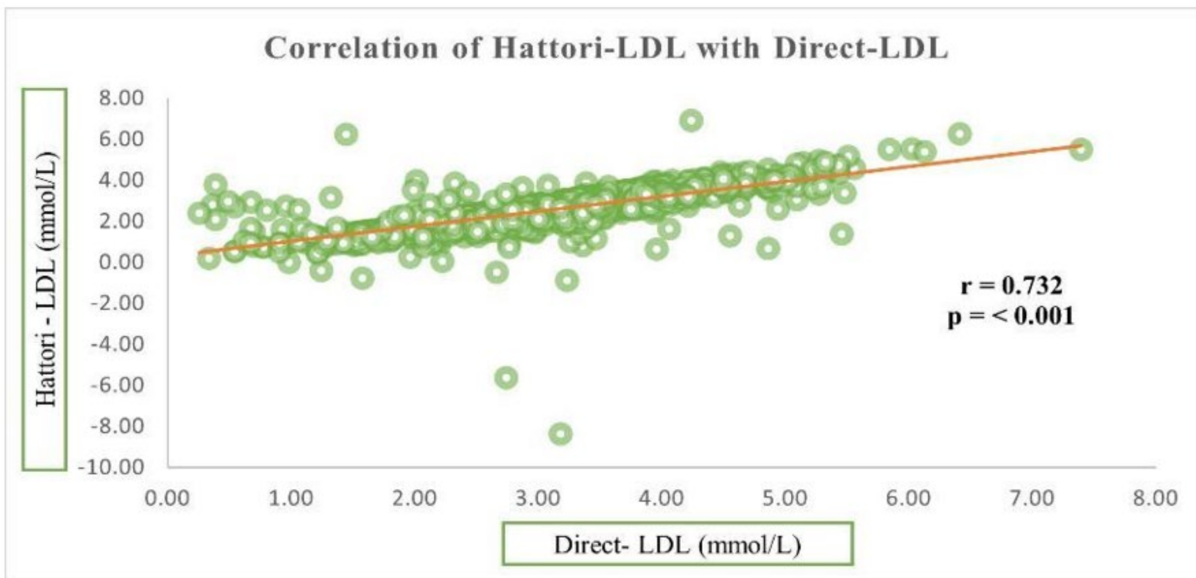


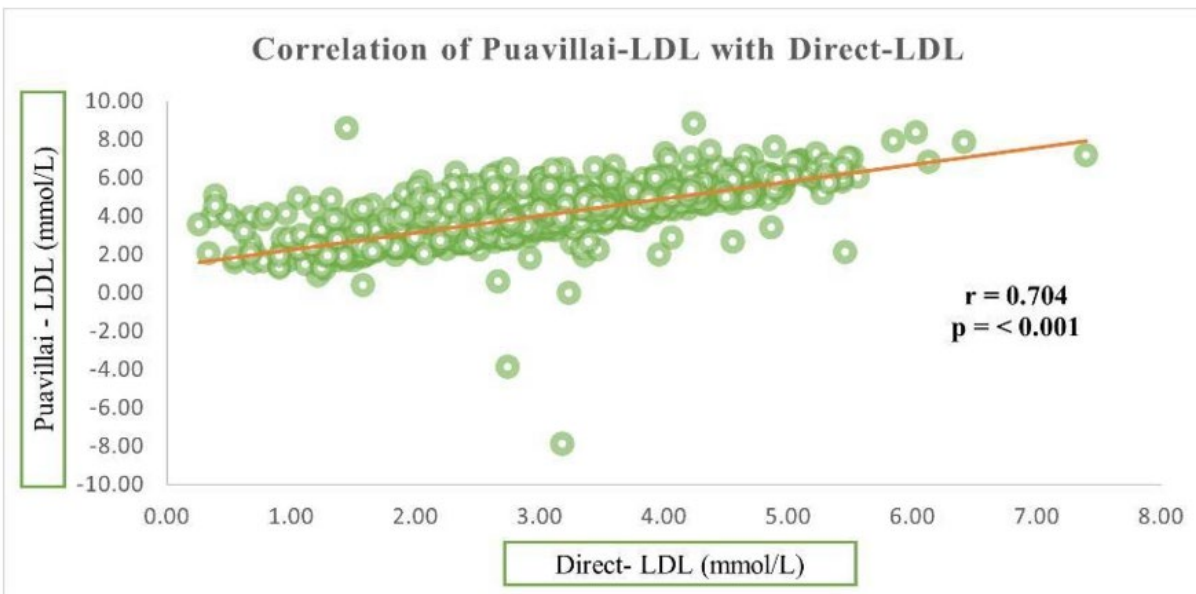

Correlation Of Anandaraja-LDL with Direct-LDL
Anandaraja- LDL (mmol/L)
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
-1.00
-2.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
Direct- LDL (mmol/L)
r = 0.796
p = < 0.001

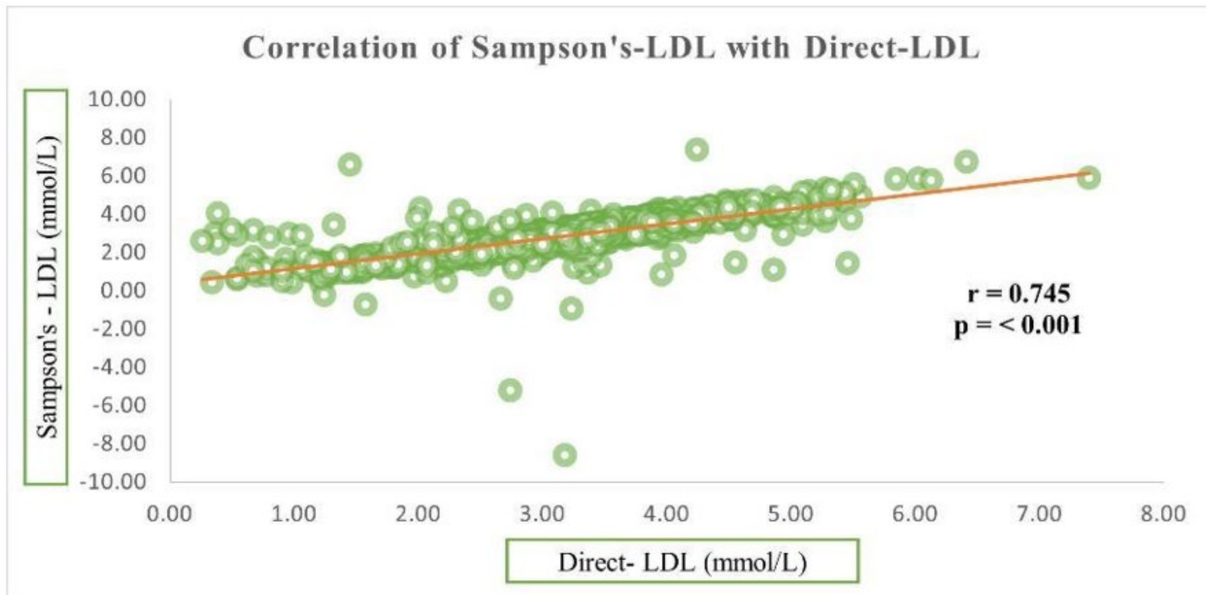

Correlation of Chen-LDL with Direct-LDL
Chen - LDL (mmol/L)
8.00
6.00
4.00
2.00
0.00
-2.00
-4.00
-6.00
-8.00
-10.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
Direct- LDL (mmol/L)
r = 0.746
p = < 0.001

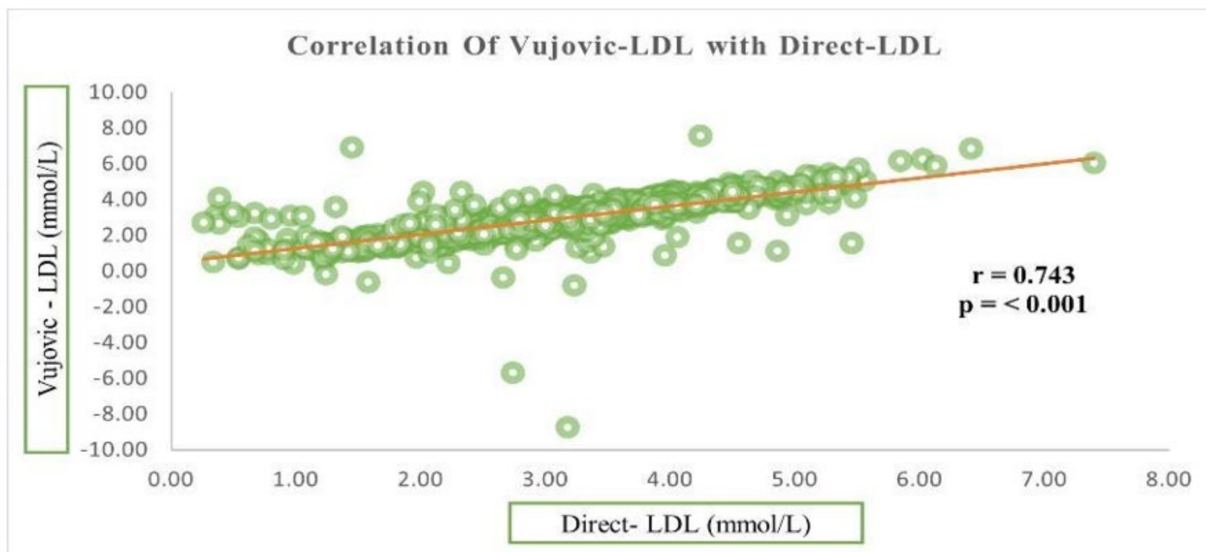

Correlation Of de Cordova-LDL with Direct-LDL
de Cordova - LDL (mmol/L)
8.00
6.00
4.00
2.00
0.00
-2.00
-4.00
-6.00
-8.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
Direct- LDL (mmol/L)
r = 0.742
p = < 0.001


Correlation of Friedewald-LDL with Direct-LDL
Friedewald - LDL (mmol/L)
10.00
8.00
6.00
4.00
2.00
0.00
-2.00
-4.00
-6.00
-8.00
-10.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
Direct- LDL (mmol/L)
r = 0.733
p = < 0.001


Correlation of Hattori-LDL with Direct-LDL
Hattori - LDL (mmol/L)
8.00
6.00
4.00
2.00
0.00
-2.00
-4.00
-6.00
-8.00
-10.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
Direct- LDL (mmol/L)
r = 0.732
p = < 0.001


Correlation of Puavillai-LDL with Direct-LDL
Puavillai - LDL (mmol/L)
10.00
8.00
6.00
4.00
2.00
0.00
-2.00
-4.00
-6.00
-8.00
-10.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
Direct- LDL (mmol/L)
r = 0.704
p = < 0.001





Correlation of various calculated formulae with direct LDL

As shown in Table 6, Surprisingly, all calculated LDL methods exhibited a strong correlation with direct LDL measurement across the study cohort. The Friedewald equation, as well as modified equations incorporating non-HDL cholesterol or apolipoprotein B, demonstrated particularly robust correlations. These findings indicate the reliability of calculated LDL methods in estimating LDL cholesterol levels. However, the mean of calculated LDL-C by all equations showed significant mean difference with directly measured LDL-C in which least mean difference (LMD) was shown by Vujovic formula and best correlation shown by Anandaraja formula

Table 6: Demographic distribution and lipid data of the study subjects.

Variable	Mean ± SD	Mean difference	t-test (Vs Direct-LDL C)	Person correlation	
				r	P
Age	51.19 ± 14.01				
Sex	532 males 543 females				
Total cholesterol (mmol/L)	4.61 ± 1.12				
Triglycerides (mmol/L)	1.67 ± 0.78				
HDL-C (mmol/L)	1.17 ± 0.52				
Direct LDL-C (mmol/L)	3.05 ± 1.00				
Comparative analysis of LDL-C by nine formula					
Ahmadi LDL-C	4.83 ± 1.56	-1.78	**<0.001**	0.554**	**<0.001**
Anandaraja LDL-C	2.74 ± 0.96	0.31	**<0.001**	**0.796****	**<0.001**
Chen LDL-C	2.71 ± 0.97	0.33	**<0.001**	0.747**	**<0.001**
de Cordova LDL-C	2.59 ± 0.85	0.46	**<0.001**	0.742**	**<0.001**
Friedewald LDL-C	2.68 ± 1.06	0.37	**<0.001**	0.733**	**<0.001**
Hattori LDL-C	2.51 ± 1.00	0.54	**<0.001**	0.733**	**<0.001**
Puavillai LDL-C	4.08 ± 1.27	-1.03	**<0.001**	0.704**	**<0.001**
Sampson's LDL-C	2.76 ± 1.05	0.29	**<0.001**	0.745**	**<0.001**
Vujovic LDL-C	2.88 ± 1.07	**0.16**	**<0.001**	**0.743****	**<0.001**

SD: Standard deviation; r=Correlation Coefficient; $p<0.05$ considered statistically significant

Estimation of LDL-C in 4 subgroups based on Age (Table 7). There were four subgroups based on age (Group 1 = <20, Group 2 = 20–39, Group 3 = 40–59 and Group 4 = ≥ 60 years). in which Ahmadi and Puavillai formulae overestimated LDL values whereas all other formulae underestimated LDL values than Direct-LDL value in all age sub-groups. LMD & good correlation was shown by Vujovic formula in all subgroups

Table 7: Distribution of calculated LDL-C in age groups <20 years, 20-39 years, 40-59 years, >=60 years.

Variable	Mean ± SD	Mean difference	t-test (Vs Direct-LDL C)	Person correlation	
				r	P
Age	**Group 1: Age = <20 (years), (n= 14)**				
Direct LDL-C	2.41 ± 1.02				
Ahmadi LDL-C	3.79 ± 1.56	-1.38	**0.002**	0.547*	**0.043**
Anandaraja LDL-C	2.16 ± 0.92	0.25	**0.049**	0.904**	**<0.001**
Chen LDL-C	2.12 ± 0.81	0.29	**0.011**	0.943**	**<0.001**
de Cordova LDL-C	2.02 ± 0.73	0.39	**0.009**	0.902**	**<0.001**
Friedewald LDL-C	2.08 ± 0.88	0.33	**0.004**	0.941**	**<0.001**
Hattori LDL-C	1.95 ± 0.83	0.46	**<0.001**	0.940**	**<0.001**
Puavillai LDL-C	3.20 ± 1.14	-0.79	**0.001**	0.799**	**0.001**
Sampson's LDL-C	2.14 ± 0.89	0.27	**0.010**	0.944**	**<0.001**
Vujovic LDL-C	2.25 ± 0.89	**0.16**	**0.092**	**0.946****	**<0.001**
	Group 2: Age = 20-39 (years), (n= 200)				
Direct LDL-C	3.01 ± 1.02				
Ahmadi LDL-C	4.77 ± 1.64	-1.76	**<0.001**	0.585**	**<0.001**
Anandaraja LDL-C	2.70 ± 0.99	0.31	**<0.001**	0.734**	**<0.001**
Chen LDL-C	2.69 ± 0.94	0.32	**<0.001**	**0.770****	**<0.001**
de Cordova LDL-C	2.56 ± 0.84	0.45	**<0.001**	0.764**	**<0.001**
Friedewald LDL-C	2.65 ± 1.01	0.36	**<0.001**	0.754**	**<0.001**
Hattori LDL-C	2.49 ± 0.95	0.52	**<0.001**	0.753**	**<0.001**
Puavillai LDL-C	4.03 ± 1.29	-1.03	**<0.001**	0.724**	**<0.001**
Sampson's LDL-C	2.73 ± 1.01	0.28	**<0.001**	0.767**	**<0.001**
Vujovic LDL-C	2.86 ± 1.03	**0.15**	**<0.001**	**0.766****	**<0.001**
	Group 3: Age = 40-59 (years), (n= 541)				
Direct LDL-C	3.12 ± 1.02				
Ahmadi LDL-C	4.97 ± 1.57	-1.85	**<0.001**	0.510**	**<0.001**
Anandaraja LDL-C	2.79 ± 0.99	0.33	**<0.001**	**0.804****	**<0.001**
Chen LDL-C	2.76 ± 1.06	0.37	**<0.001**	0.699**	**<0.001**
de Cordova LDL-C	2.64 ± 0.91	0.49	**<0.001**	0.696**	**<0.001**
Friedewald LDL-C	2.71 ± 1.18	0.42	**<0.001**	0.687**	**<0.001**
Hattori LDL-C	2.54 ± 1.10	0.59	**<0.001**	0.687**	**<0.001**
Puavillai LDL-C	4.17 ± 1.32	-1.05	**<0.001**	0.663**	**<0.001**
Sampson's LDL-C	2.80 ± 1.15	0.32	**<0.001**	0.698**	**<0.001**
Vujovic LDL-C	2.92 ± 1.17	**0.20**	**<0.001**	**0.696****	**<0.001**
	Group 4: Age = >=60 (years), (n= 320)				
Direct LDL-C	**2.97 ± 0.95**				
Ahmadi LDL-C	**4.67 ± 1.45**	-1.70	**<0.001**	0.600**	**<0.001**
Anandaraja LDL-C	**2.70 ± 0.88**	0.28	**<0.001**	0.815**	**<0.001**
Chen LDL-C	**2.68 ± 0.82**	0.29	**<0.001**	**0.836****	**<0.001**
de Cordova LDL-C	**2.54 ± 0.74**	0.43	**<0.001**	0.819**	**<0.001**
Friedewald LDL-C	**2.66 ± 0.88**	0.31	**<0.001**	0.830**	**<0.001**
Hattori LDL-C	**2.49 ± 0.83**	0.48	**<0.001**	0.829**	**<0.001**

Puavillai LDL-C	**3.99 ± 1.14**	-1.02	**<0.001**	0.763**	**<0.001**
Sampson's LDL-C	**2.74 ± 0.88**	0.24	**<0.001**	**0.836****	**<0.001**
Vujovic LDL-C	**2.86 ± 0.90**	**0.11**	**<0.001**	**0.836****	**<0.001**

SD: Standard deviation; r=Correlation Coefficient; p<0.05 considered statistically significant

Estimation of LDL-C in 4 subgroups based on TG ranges (Table 8). Since we had only 3 values whose TG was > 4.51 mmol/dL, we removed these readings from database so we had only 4 sub-groups Group 1: TG <0.56 mmol/L, Group 2: TG 0.56-1.69 mmol/L, Group 3: TG = 1.70-3.38 mmol/L & Group 4: TG = 3.39-4.51 mmol/L

Table 8: Estimation of LDL-C in 4 subgroups based on TG ranges (<0.56, 0.56–1.69, 1.70–3.38, 3.39–4.51 and > 4.51 mmol/L).

Variable	Mean ± SD	Mean difference	t-test (Vs Direct-LDL C)	Person correlation	
				r	P
	Group 1: TG <0.56 (mmol/L), (n=15)				
Direct LDL-C	2.05 ± 0.67				
Ahmadi LDL-C	2.21 ± 0.62	-0.16	0.002	0.723**	0.002
Anandaraja LDL-C	1.97 ± 0.77	**0.08**	**<0.001**	**0.851****	**<0.001**
Chen LDL-C	1.72 ± 0.64	0.33	**<0.001**	0.800**	**<0.001**
de Cordova LDL-C	1.53 ± 0.54	0.52	**<0.001**	0.792**	**<0.001**
Friedewald LDL-C	1.81 ± 0.71	0.24	**<0.001**	0.807**	**<0.001**
Hattori LDL-C	1.70 ± 0.67	0.35	**<0.001**	0.807**	**<0.001**
Puavillai LDL-C	2.22 ± 0.72	-0.17	**0.001**	0.778**	**0.001**
Sampson's LDL-C	1.77 ± 0.74	0.28	**<0.001**	0.805**	**<0.001**
Vujovic LDL-C	1.87 ± 0.71	0.18	**<0.001**	0.803**	**<0.001**
	Group 2: TG 0.56-1.69 (mmol/L), (n=630)				
Direct LDL-C	2.95 ± 1.00				
Ahmadi LDL-C	4.04 ± 1.07	-1.09	**<0.001**	0.711**	**<0.001**
Anandaraja LDL-C	2.76 ± 0.93	0.19	**<0.001**	**0.818****	**<0.001**
Chen LDL-C	2.61 ± 0.95	0.34	**<0.001**	0.739**	**<0.001**
de Cordova LDL-C	2.40 ± 0.81	0.55	**<0.001**	0.745**	**<0.001**
Friedewald LDL-C	2.66 ± 1.04	0.29	**<0.001**	0.732**	**<0.001**
Hattori LDL-C	2.49 ± 0.98	0.46	**<0.001**	0.732**	**<0.001**
Puavillai LDL-C	3.65 ± 1.11	-0.7	**<0.001**	0.747**	**<0.001**
Sampson's LDL-C	2.70 ± 1.05	0.25	**<0.001**	0.740**	**<0.001**
Vujovic LDL-C	2.80 ± 1.05	**0.15**	**<0.001**	**0.737****	**<0.001**
	Group 3: TG = 1.70-3.38 (mmol/L), (n=376)				
Direct LDL-C	3.24 ± 0.96				
Ahmadi LDL-C	5.80 ± 1.08	-2.56	**<0.001**	0.617**	**<0.001**
Anandaraja LDL-C	2.77 ± 0.98	0.47	**<0.001**	**0.790****	**<0.001**
Chen LDL-C	2.91 ± 0.98	0.34	**<0.001**	0.735**	**<0.001**
de Cordova LDL-C	2.85 ± 0.82	0.39	**<0.001**	0.733**	**<0.001**
Friedewald LDL-C	2.77 ± 1.09	0.47	**<0.001**	0.731**	**<0.001**
Hattori LDL-C	2.60 ± 1.03	0.65	**<0.001**	0.730**	**<0.001**
Puavillai LDL-C	4.65 ± 1.11	-1.40	**<0.001**	0.719**	**<0.001**
Sampson's LDL-C	2.90 ± 1.04	0.34	**<0.001**	0.737**	**<0.001**

Vujovic LDL-C	3.05 ± 1.09	**0.19**	**<0.001**	**0.734****	**<0.001**
	Group 4: TG = 3.39-4.51 (mmol/L) , (n=54)				
Direct LDL-C	3.08 ± 1.12				
Ahmadi LDL-C	7.98 ± 1.01	-4.90	**<0.001**	0.734**	**<0.001**
Anandaraja LDL-C	2.41 ± 1.07	0.67	**<0.001**	0.757**	**<0.001**
Chen LDL-C	2.90 ± 0.93	0.18	0.07	0.758**	**<0.001**
de Cordova LDL-C	3.14 ± 0.79	**-0.06**	0.54	**0.760****	**<0.001**
Friedewald LDL-C	2.45 ± 1.02	0.63	**<0.001**	0.754**	**<0.001**
Hattori LDL-C	2.29 ± 0.96	0.79	**<0.001**	0.754**	**<0.001**
Puavillai LDL-C	5.61 ± 1.09	-2.54	**<0.001**	**0.760****	**<0.001**
Sampson's LDL-C	2.70 ± 0.93	0.37	**<0.001**	0.754**	**<0.001**
Vujovic LDL-C	2.92 1.03	0.16	0.12	0.756**	**<0.001**

TG: Triglycerides; SD: Standard deviation; r=Correlation Coefficient; p<0.05 considered statistically significant

Overestimation of LDL was shown by Ahmadi, Puavillai in all TG subgroups while reverse ie underestimation of LDL was shown by all others except de Cordova which showed underestimated LDL at TG < 3.38 mmol/L & overestimation was seen at TG >3.38 mmol/L. LMD & best correlation was shown by Anandaraja formula at TG < 0.56 mmol/L. Vujovic formula showed LMD & good correlation at TG levels in between 0.56-3.38 mmol/L and best correlation at this level was shown by Anandaraja formula which was little higher than Vujovic formula. LMD & best correlation was shown by de Cordova formula at TG > 3.38 mmol/L. Puavillai formula also showed best correlation although it had significant mean difference

Estimation of LDL-C in 3 subgroups based on TC ranges (Table 9a, 9b)

We had 3 subgroups Group 1: TC = < 5.17 mmol/L, Group 2: TC 5.17-6.18 mmol/L & Group 3: TG = > 6.18 mmol/L, in which Ahmadi and Puavillai formulae overestimated LDL values whereas all other formulae underestimated LDL values than Direct-LDL value in all TC sub-groups. LMD & good correlation was shown by Vujovic formula in all subgroups of TC except subgroup 2 where best corelation was seen while Anandaraja showed best correlation ('r' = 0.659 and 'r' = 0.338 in subgroups 1 and 3). Very poor correlation was shown by Ahmadi formula at TC >5.17 mmol/L.

Table 9a: Estimation of LDL-C in 3 subgroups based on TC ranges < 5.17 mmol/L, 5.17-6-18 mmol/L and > 6.18 mmol/L.

Variable	**Mean ± SD**	**Mean difference**	**t-test (Vs Direct-LDL C)**	**Person correlation**	
				r	**P**
	Group 1: Total cholesterol <5.17 (mmol/L), (n= 750)				
Direct LDL-C	2.63 ± 0.77				
Ahmadi LDL-C	4.25 ± 1.29	-1.63	**<0.001**	0.360**	**<0.001**
Anandaraja LDL-C	2.29 ± 0.70	0.34	**<0.001**	**0.659****	**<0.001**
Chen LDL-C	2.28 ± 0.76	0.34	**<0.001**	0.543**	**<0.001**
de Cordova LDL-C	2.20 ± 0.66	0.42	**<0.001**	0.542**	**<0.001**
Friedewald LDL-C	2.22 ± 0.86	0.41	**<0.001**	.0526**	**<0.001**
Hattori LDL-C	2.08 ± 0.80	0.55	**<0.001**	0.525**	**<0.001**
Puavillai LDL-C	3.52 ± 0.99	-0.90	**<0.001**	0.506**	**<0.001**
Sampson's LDL-C	2.30 ± 0.83	0.32	**<0.001**	0.545**	**<0.001**
Vujovic LDL-C	2.41 ± 0.85	**0.21**	**<0.001**	**0.538****	**<0.001**
Table 9b: Estimation of LDL-C in 3 subgroups based on TC ranges < 5.17 mmol/L, 5.17-6-18 mmol/L and > 6.18 mmol/L.					
	Group 2: Total cholesterol = 5.17-6.18 (mmol/L), (n= 244)				
Direct LDL-C	3.77 ± 0.63				
Ahmadi LDL-C	5.84 ± 1.12	-2.06	**<0.001**	-0.025	0.699

Anandaraja LDL-C	3.52 ± 0.40	0.26	<0.001	0.378**	<0.001
Chen LDL-C	3.47 ± 0.35	0.30	<0.001	0.504**	<0.001
de Cordova LDL-C	3.26 ± 0.30	0.51	<0.001	0.402**	<0.001
Friedewald LDL-C	3.47 ± 0.45	0.30	<0.001	0.498**	<0.001
Hattori LDL-C	3.25 ± 0.43	0.52	<0.001	0.497**	<0.001
Puavillai LDL-C	5.06 ± 0.57	-1.28	<0.001	0.189**	0.003
Sampson's LDL-C	3.57 ± 0.42	0.21	<0.001	0.501**	<0.001
Vujovic LDL-C	3.70 ± 0.41	0.07	0.051	0.509**	<0.001
	Group 3: Total cholesterol = >6.18 (mmol/L), (n= 81)				
Direct LDL-C	4.75 ± 0.82				
Ahmadi LDL-C	7.11 ± 1.29	-2.36	<0.001	-0.053	0.636
Anandaraja LDL-C	4.51 ± 0.65	0.23	0.016	0.338**	0.002
Chen LDL-C	4.43 ± 0.61	0.32	0.001	0.301**	0.006
de Cordova LDL-C	4.11 ± 0.51	0.63	<0.001	0.237*	0.033
Friedewald LDL-C	4.48 ± 0.51	0.27	0.009	0.328**	0.003
Hattori LDL-C	4.20 ± 0.68	0.54	<0.001	0.329**	0.003
Puavillai LDL-C	6.30 ± 0.81	-1.55	<0.001	0.120	0.288
Sampson's LDL-C	4.56 ± 0.70	0.19	0.055	0.333**	0.002
Vujovic LDL-C	4.75 ± 0.69	0.00	0.990	0.315**	0.004

TC: Total cholesterol; SD: Standard deviation; r=Correlation Coefficient; p<0.05 considered statistically significant

Estimation of LDL-C in 3 subgroups based on HDL ranges (Table 10).

We had 3 subgroups Group 1: HDL = < 1.03 mmol/L, Group 2: TC 1.03-1.52 mmol/L & Group 3: TG = > 1.52 mmol/L, in which Ahmadi and Puavillai formulae overestimated LDL values whereas all other formulae underestimated LDL values than Direct-LDL value in all HDL sub-groups except Anandaraja formula which showed underestimation of LDL values at HDL < 1.52 mmol/L & overestimated at HDL >1.52 mmol/L. LMD & good correlation was shown by Vujovic formula at HDL < 1.52 mmol/L. Best correlation was exhibited by Chen formula at HDL < 1.52 mmol/L. LMD & best correlation was shown by Anandaraja at HDL > 1.52 mmol/L.

Table 10: Estimation of LDL-C in 3 subgroups based on HDL ranges <1.03 mmol/L, 1.03-1.52 mmol/L and >1.53 mmol/L.

Variable	Mean ± SD	Mean difference	t-test (Vs Direct-LDL C)	Person correlation	
				r	P
	Group 1: Total cholesterol <5.17 (mmol/L), (n= 750)				
Direct LDL-C	2.75 ± 1.00				
Ahmadi LDL-C	4.87 ± 1.50	-2.11	<0.001	0.545**	<0.001
Anandaraja LDL-C	2.15 ± 0.90	0.60	<0.001	0.803**	<0.001
Chen LDL-C	2.45 ± 0.86	0.31	<0.001	0.812**	<0.001
de Cordova LDL-C	2.40 ± 0.76	0.36	<0.001	0.799**	<0.001
Friedewald LDL-C	2.34 ± 0.94	0.42	<0.001	0.795**	<0.001
Hattori LDL-C	2.19 ± 0.89	0.56	<0.001	0.795**	<0.001
Puavillai LDL-C	3.90 ± 1.15	-1.15	<0.001	0.738**	<0.001
Sampson's LDL-C	2.44 ± 0.93	0.31	<0.001	0.807**	<0.001
Vujovic LDL-C	2.57 ± 0.95	0.19	<0.001	0.808**	<0.001
	Group 1 : HDL = < 1.03 (mmol/L), (n=340)				
Direct LDL-C	3.16 ± 0.95				

Ahmadi LDL-C	4.89 ±1.51	-1.73	**<0.001**	0.595**	**<0.001**
Anandaraja LDL-C	2.94 ± 0.82	0.22	**<0.001**	0.797**	**<0.001**
Chen LDL-C	2.87 ± 0.83	0.29	**<0.001**	**0.813****	**<0.001**
de Cordova LDL-C	2.70 ± 0.75	0.46	**<0.001**	0.797**	**<0.001**
Friedewald LDL-C	2.86 ± 0.89	0.30	**<0.001**	0.804**	**<0.001**
Hattori LDL-C	2.68 ± 0.83	0.48	**<0.001**	0.804**	**<0.001**
Puavillai LDL-C	4.22 ± 1.17	-1.06	**<0.001**	0.745**	**<0.001**
Sampson's LDL-C	2.93 ± 0.89	0.23	**<0.001**	0.811**	**<0.001**
Vujovic LDL-C	3.06 ± 0.91	**0.10**	**<0.001**	**0.812****	**<0.001**
	Group 3: HDL = >1.53 (mmol/L), (n= 100)				
Direct LDL-C	3.32 ± 1.12				
Ahmadi LDL-C	4.28 ± 1.92	-0.96	**<0.001**	0.596**	**<0.001**
Anandaraja LDL-C	3.41 ±1.02	**-0.08**	**0.188**	**0.832****	**<0.001**
Chen LDL-C	2.65 ± 1.70	0.67	**<0.001**	0.541**	**<0.001**
de Cordova LDL-C	2.48 ± 1.43	0.85	**<0.001**	0.565**	**<0.001**
Friedewald LDL-C	2.67 ± 1.89	0.65	**<0.001**	0.520**	**<0.001**
Hattori LDL-C	2.50 ± 1.77	0.82	**<0.001**	0.519**	**<0.001**
Puavillai LDL-C	3.81 ± 1.94	-0.49	**0.002**	0.591**	**<0.001**
Sampson's LDL-C	2.75 ± 1.84	0.57	**<0.001**	0.538**	**<0.001**
Vujovic LDL-C	2.84 ± 1.89	0.49	**0.003**	0.533**	**<0.001**

HDL : High Density lipoprotein; SD: Standard deviation; r=Correlation Coefficient; p<0.05 considered statistically significant

Discussion

Serum LDL-C level not only plays a crucial role in development of atherosclerosis which is proved to be a well-known factor in development of coronary heart disease but it also plays a role assessing the treatment session of these patients [1,3]. Estimation of LDL to a very precise level is therefore necessary but a difficult task when direct LDL measurement facility is not available in the lab setup. To overcome this situation many formulae have been developed and surprisingly they show a good positive correlation with direct LDL measurement just like this and other studies [11,12,16,22-26]. In this study the study population was subdivided into various subgroups based on age, TG, TC and HDL levels to validate 9 different formulae. Most of these formulae showed good correlation with D-LDL in between the subgroups. In this study Vujovic formula came out to show least mean difference and good correlation in various subgroups based on different criterias when compared to routinely used Friedewald formula which is in line with Vujovic et al. study in Serbian population and Wadhwa N and Krishnaswamy R study in Indian population. In Wadhwa study Vujovic formula came out to the best at all levels of TG, but in our study at lower TG level ie <0.56 mmol/L Anandaraja formula showed the best correlation which might be due to lesser no of individuals in this subgroup and at higher TG level > 3.38 de Cordova along with Puavillai showed best correlation.

In this study after Vujovic formula some other formulae like Anandaraja, Chen, de Cordova, Puavillai showed best correlation in one or other subgroups. Friedewald formula which is used routinely cannot be used at higher TG, higher total cholesterol or lower HDL levels [27,28].

Different studies are conducted to evaluate effectiveness of formulae alternative to direct LDL estimation by comparing one to two formulae with direct and commonly used Friedewald formula.

Most of these studies evaluated one or two formulae with very few taking more than two formulae like our study in which we compared and correlated 9 formulae. Also the study population was subdivided into various subgroups based on age, TG, TC and HDL levels in our study but the study population was grouped based on TG levels in most of the studies.

The major findings of different studies is listed in Table 11.

Table 11: Major findings of different studies.

Name of the study using author's name	Area of studied population	Comparison of Friedewald formula with newer formula which is modified Friedewald formula
Sha MFR et al [12]	Bangladeshi	Regression equation is more accurate to D-LDL when compared with Friedewald
de Cordova [8]	Brazilian	de Cordova formula is better than Friedewald formula
Ahmadi [5]	Iranian	Ahmadi formula is better at lower TG values
Gupta et al [22]	Indian	Friedewald formula is better than Anandaraja formula
Anandaraja et al [6]	Indian	Anandaraja formula is better in Indian population
Puavillai et al [10]	Thailand	Puavillai formula is better than Friedewald formula
Vujovic et al [11]	Serbian	Vujovic formula is better than Friedewald
Wadhwa et al [29]	Indian	Vujovic Formula is better than any other formula for Indian population which is similar to our study
Hattori et al [9]	Japanese	Hattori formula is better than Friedewald
Garule et al [30]	Indian	Puavillai formula is better than any other in Indian population at most TG levels but best is different for different TG levels
Karkhaneh et al [31]	Iranian	Here groups were divided based on other biochemical parameters of lipid profile too just like our study With difference in formula that came out to be best alternative to D-LDL was Hattori and de Cordova and our study was Vujovic
Krishnaveni et al [32]	Indian	Friedewald formula correlated maximally with D-LDL at all TG levels except < 100mg/dL where Anandaraja formula is better
Teerakanchana et al [33]	Thailand	Friedewald Formula gave inconsistent results at different level of TGs when compared to D-LDL
Sahu et al [34]	Indian	Friedewald formula gave inconsistent result still remains the choice after D-LDL due to cost effectiveness in country like India
Warade et al [35] **Sudha et al [36]**	Indian	D-LDL assay should be considered as and when possible due to variability in results with commonly used Friedewald formula

Limitations

Though the sample size was good enough overall when the population was subdivided into subgroups some of them had a very low data. The study compared and correlated various formulae of LDL-C with direct assay of LDL by only one method and no ultracentrifugation or precipitation was done which is known as reference method. Individuals having age group < 20 , TG < 0.56 and > 4.51 mmol/L were very less so there are chances of bias. > 4.51 mmol/L of TG level data was very in significant and so was excluded. Total cholesterol at higher level >6.18 mmol/L was seen in only 7.5% of whole population which is again low to increase chance of bias. Also, HDL > 1.53 mmol/L was seen in 9.3% individuals again small number of samples. One possibility of not getting higher level of TG, TC or HDL is that patients were on treatment with statins. Lastly only 9 formulae were considered for the study which omitted other formulae which could have given different result.

Conclusion

We are in favour of Vujovic formula for Indian population as it looked like a better alternative when compared with most commonly used Friedewald formula and other formulae. However more studies using more sample size particularly taking lower TG and higher TG levels into consideration, and from different ethnicities and geographical areas must be done to be able to use the above method confidently in Indian population.

Abbreviations

Chol: Cholesterol; FBS: Fasting blood sugar; HDL: High-density lipoprotein; LDL-C: Low-density lipoprotein-cholesterol; TC: Total cholesterol; TG: Triglyceride

Acknowledgements

We are thankful to the SMCH institute for allowing us to proceed for the above work.

Special thanks to Dr Gautham for providing with valuable suggestions regarding various graphical plots to be used

Authors' contributions

RK– research study plan. KDS, RKPK, BP research data collection. MN, AS Data analysis, statistical work and manuscript preparation. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

Funding

NIL.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1975 Helsinki declaration as revised in 2008. This study was approved by the Ethics Committee of Srinivasan Medical College and Hospital (IEC No. 18/2022).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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Review Article

MicroRNA Significance in Cancer: An Updated Review on Diagnostic, Prognostic, and Therapeutic Perspectives.

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Keywords
Cancer, microRNA(miRNA), Tumor-Suppressor Genes, Polymerase Chain Reaction (PCR), Next Generation Sequencing (NGS), Microarray, Antisense Oligonucleotides (ASOs)

Abstract

Abstract
The article provides a thorough and up-to-date analysis of the role that microRNAs (miRNAs) within the realm of cancer therapy, paying specific attention to their diagnostic, prognostic as well as therapeutic capabilities. The miRNAs (small non-coding RNAs) are the current major genes that regulate gene expression. They are a key factor in the genesis of cancer. They are oncogenes, or tumor suppressors that play key functions in the signaling pathway that contribute to the development of cancer. This article focuses on the double importance of microRNAs for cancer oncogenesis. This includes both their ability to inhibit cancer suppressor genes and the stimulation of cancer-causing oncogenes. MicroRNAs have been identified for a long time as biomarkers to help in diagnosing cancer and have distinct signatures specific to different kinds of cancer. There are many detection strategies including RT-qPCR, Next Generation Sequencing (NGS) as well as Microarray Analysis that have been evaluated to prove their effectiveness in aiding the non-invasive diagnosis of cancer. The paper provides an overview of the importance of miRNAs to prognosis, highlighting their ability to forecast tumor progression as well as outcomes for cancer patients. In addition, their therapeutic value remains a subject of research. Research is being conducted in order to investigate miRNA-targeting therapy including antisense oligonucleotides, or small molecules inhibitors as possible treatment options for cancer. These methods could favor more specific and individualized approaches than the current techniques. The article also focuses on the current challenges and future prospects linked to miRNA research and demonstrates the complex biological functions they play as well as clinical applications that require investigation. The review is the source of information for researchers, clinicians and scientists who are interested in advancing studies into cancer research as well as personalized treatments.

Introduction

Cancer poses a worldwide health crisis that impacts millions annually. Although advances have been made in treatments and management approaches for cancer detection, and management remains complex tasks that often necessitate

comprehensive approaches. Over the last ten years, great strides have been made toward understanding molecular mechanisms underlying cancer progression as part of cancer research with microRNA (miRNA) serving as an increasingly researched topic. MicroRNAs are non-coding RNA molecules that play an essential part in gene regulation, play an integral part. It is comprised of 22 nucleotides per microRNA molecule and bound directly with specific messenger RNA (mRNA), these non-coding molecules precisely manage gene expression levels by binding directly with messenger RNA (mRNA). The adhesions between molecules could potentially impede translation processes; miRNAs utilize this mechanism to regulate the expression of many target genes and influence various cellular activities - providing significant gene regulatory capabilities. miRNAs function as posttranscriptional regulators by binding to messenger RNA molecules' 3' untranslated regions (UTR), leading either to degradation or translational repression thereby fine-tuning gene expression levels and providing more precise control of gene expression levels. The miRNAs modulate gene expression through sequence-specific targeting of multiple messenger RNA (mRNA) molecules, any given messenger RNA could potentially be targeted by multiple miRNAs; their biogenesis and processing involve many enzymes and regulatory proteins to ensure functional miRNA production [1]. The miRNA binding to target mRNAs can alter biological processes and lead to various diseases, making miRNAs useful biomarkers in diagnosing disease diagnosis, prognosis, and prognosticate purposes, with some specific miRNAs possessing therapeutic potential against specific conditions [2].

The miRNA molecules have been discovered to regulate certain target mRNAs, thus altering important physiological processes like cell proliferation, differentiation, and apoptosis. Furthermore, abnormal miRNA expression patterns have been linked with diseases like cancer that contribute to tumor development and progression [3]. The multifaceted role of Vitamin D in disease prevention and cure, as elucidated in recent studies, highlights its potential therapeutic significance in oncology, emphasizing the need for further research into its molecular mechanisms and health benefits [4]. This article intends to present an updated account of the importance of miRNAs for cancer diagnosis, prognostication, and therapeutic development. We aim to gather the latest research findings and advancements to gain an understanding of their possible effects on clinical practice. Additionally, we will investigate challenges and future directions associated with harnessing miRNAs to maximize cancer management potential.

Role of MicroRNAs in Cancer Development

MicroRNAs (miRNAs) exert a substantial influence on the development of cancer by participating in many pathways, including the suppression of tumor-suppressor genes and the activation of oncogenes [5].

Suppression of Tumor-Suppressor Genes

MicroRNAs (miRNAs) play an essential role as tumor suppressor genes by down-regulating specific target genes involved with cell proliferation, apoptosis, and differentiation processes. These inhibitors work by binding to sequences present on target messenger RNA (mRNA), leading to its cleavage, translational repression, or deadenylation before its subsequent degradation [6]. Many microRNAs have been discovered for their tumor-suppressing capabilities by targeting pathways and genes involved in cancer formation. MiR-34 family transcription factors, which are under the control of p53 tumor suppressor gene regulation, downregulate many genes such as Cyclin D1, E2, CDK4, CDK6, Myc, and BCl2, leading to cell cycle arrest and apoptosis, thereby curbing tumor growth and inhibiting tumor spread. MiR-15 and miR-16 clusters, commonly found deleted in chronic lymphocytic leukemia (CLL), targets the anti-apoptotic BCL2 gene to induce apoptosis and prevent tumorigenesis. Let-7 family targets Ras and Myc oncogenes to prevent cell cycle progression, and proliferation and induce apoptosis in various cancer types such as lung, breast, gastric colon, and prostate cancers. MiR-200 family miRNAs, particularly miR-200c, have been found to target ZEB1 and ZEB2, in turn suppressing epithelial-to-mesenchymal transition (EMT), thus inhibiting cancer metastasis, leading to less aggressive metastatic properties in cancers like breast and non-small cell lung cancer (NSCLC). Their actions demonstrate how miRNAs serve as tumor suppressors whose deregulation could significantly halt cancer progression thereby underscoring the therapeutic potential of miRNA-based interventions as tumor suppressors [7,8].

Activation of Oncogenes

MicroRNAs (miRNAs) play an essential role in tumor suppression by targeting and downregulating the expression of various tumor suppressor genes, but they may also act as oncogenes by directly down-regulating certain oncoprotein genes that prevent tumorigenesis. MiR-17-92 cluster, commonly referred to as "oncomir-1," targets and inhibits tumor suppressor proteins like PTEN, p21, and E2F; ultimately leading to increased cell proliferation, survival, and angiogenesis. MiR-21 also targets PTEN, PDCD4, and TIMP3, activating survival-supportive pathways like PI3K/Akt and MAPK which promote tumor growth, invasion, and metastasis. miR-155 downregulates TP53INP1, SOCS1, and SHIP1, thus increasing cell proliferation, survival, and immune evasion. MiR-372/373 cluster targets the LATS2 tumor suppressor and activates transcriptional co-activators known as YAP/TAZ that drive proliferation and stemness pathways. Deregulation of these oncogenic miRNAs either through upregulation or genetic alteration contributes significantly to cancer development/progression highlighting them as therapeutic targets within cancer treatment protocols [7,9,10].

Impact on Signalling Pathways

MicroRNAs (miRNAs) play an essential role in cancer development through various mechanisms, from epigenetic regulation of miRNAs to directly altering signaling pathways

that drive cancer development. Epigenetic modifications such as DNA methylation or histone modifications can alter miRNA expression patterns to suppress or overexpress specific miRNAs, disrupting normal signaling pathways and potentially contributing to cancer formation and progression. The miRNAs play an essential role in cancer cells activity and regulation by modulating key signaling pathway components, targeting oncogenes or tumor suppressor genes, participating in feedback loops, modulating crosstalk, and experiencing epigenetic modifications - these miRNA-driven changes impact signaling pathways greatly and play an integral part in cancer cell processes such as abnormal growth, survival, invasion, and metastasis.

In one such study, researchers have discovered that miRNA-21 disrupts TNF receptor 1 (TNFR1) signaling while stimulating its counterpart (TNFR2) in cervical cancer [11]. Similar evidence exists with regards to miRNAs used to manage Hepatocellular Carcinoma (HCC), specifically controlling cell proliferation, invasion, metastasis, and drug sensitivity by manipulating key signaling pathways like PTEN/PI3K/Akt, Hippo-YAP/TAZ, and Wnt/b-catenin [12]. miRNA-425-5p promotes breast cancer growth via activating its activating signaling pathway while miR-9 suppresses its growth [13]. Within pancreatic ductal adenocarcinoma (PDAC), several microRNAs, including miR-217, have been identified as key regulators of the KRAS signaling pathway. miRNA-222 regulates Capan-2 pancreatic cancer cell line growth by specifically targeting P57 [7,14].

MicroRNAs as Diagnostic Biomarkers

MicroRNAs offer several distinct advantages as biomarkers over more traditional approaches for early disease diagnosis, including cancer detection. Their secretions into circulation remain stable over time making miRNAs ideal tools. Early diagnosis is key to improving patient prognosis and limiting treatment options, and microRNAs hold immense promise as multimarker models for accurate diagnoses, targeted therapy approaches, and tracking treatment response evaluation [15]. Circulating miRNAs and exosomal miRNAs may provide a more comprehensive understanding of disease processes while improving diagnostic accuracy. They have been discovered in blood, urine, and saliva samples taken from various body fluids. The miRNAs can provide non-invasive sampling that's ideal for cases when more invasive procedures, like biopsy, are either impractical or too inaccurate to use accurately. miRNA expression profiles can help pinpoint cancer origin and subtype for tailored personalized treatment plans and outcomes prediction; or predict individual responses to drugs which allows for optimizing treatment regimens while mitigating side effects [16,17].

Reverse transcription followed by quantitative polymerase chain reaction (RT-qPCR)

Reverse transcription converts microRNAs to complementary DNAs using reverse transcription technology before amplifying and quantifying them using specific primers tailored for miRNA quantification amplification and quantification [18]. With accurate yet sensitive quantification capabilities of miRNA levels quantitatively, RT-qPCR serves as an indication or prognostic biomarker in various malignancies. RT-qPCR offers another non-invasive diagnosis method that is easily available, making this approach viable for use when diagnosing issues related to blood, urine, and saliva. RT-qPCR offers many advantages over alternative approaches for miRNA detection, including its ability to simultaneously identify multiple miRNAs simultaneously allowing multimarker models for accurate diagnosis and treatment; however, its use may come with risks. These considerations include selecting an analysis platform, taking note of any preanalytical requirements necessary, and understanding their effects on miRNA expression [19,20]. Analytical challenges associated with using miRNAs as diagnostic biomarkers include the need to account for batch effects between laboratories [21]. Therefore, RT-qPCR has emerged as one of the key techniques for detecting and quantifying miRNAs that serve as biomarkers in clinical diagnostic tests.

Next-Generation Sequencing (NGS)

MicroRNAs have demonstrated promise as cancer diagnostic markers when examined through Next-Generation Sequencing (NGS). NGS allows rapid sequencing of millions of DNA fragments simultaneously, providing accurate detection and profiling of microRNAs throughout their entire sequence. Researchers may use microRNA sequencing on cancer tissue or biofluid samples to isolate signatures associated with various forms of cancer and use this information for diagnosis, classification, and stratification purposes among cancer patients. There have been multiple studies that focus on the significance of miRNA in cancer. One such research paper utilized NGS technology to compare miRNA expression levels between lung cancer patients and healthy controls using serum samples from their bodies. The miRNA analyses provide powerful markers for cancer detection. Profiling cancer requires closely inspecting tissue sample expression patterns for purposes of diagnosing and prognosticating cancer based on crucial data analysis techniques. As technology progresses and data analytics techniques mature further, miRNA may prove itself invaluable as both an invaluable diagnostic tool in research settings as well as clinical practice settings. NGS analyses of miRNAs offer powerful markers for cancer detection [22,23].

Microarray Analysis

Microarray analysis uses miRNA-specific probes anchored to a solid surface to quickly and simultaneously assess multiple miRNA expression levels at once making this efficient means for miRNA profiling. Following hybridization to the chip, scanning, and analysis take place to detect differentially expressed miRNAs. Microarray analysis is an established technique for studying microRNA expression patterns as well as their roles in disease. Microarray profiling of miRNA expression

involves using a microarray chip containing probes for specific miRNAs to hybridize with labeled RNA samples and detect and analyze hybridization signals that occur between them and detected miRNA molecules [24]. Microarray analysis offers several distinct advantages when it comes to miRNA detection, including being able to simultaneously identify multiple miRNAs at once and detect them across body fluids - making this a non-invasive yet convenient diagnosis method. Multiple research projects utilizing miRNA as a diagnostic tool in microarray analysis are underway; in one such study, miRNA array analysis and bioinformatics methods were utilized to analyze miRNA expression profiles from pancreatic cancer tissue samples [25].

Specific miRNA Signatures in Different Cancer Types:

The miRNA signature includes an extensive set of microRNAs (miRNAs) with diagnostic, therapeutic, and prognostic potential across numerous diseases. miRNA-21 can be targeted as part of cancer therapy [26], miRNA-34 serves to treat Alzheimer's and predict its progression, while miRNA-122 helps diagnose liver diseases related to cholesterol and lipid metabolism. miRNA-155 modulates immune response; miRNA-125b serves as an early cancer detection indicator, miRNA-29 has been associated with increased severity of fibrosis; miRNA-146a signals neuroinflammation while let-7 predicts survival among cancer patients, MiR-223 levels are high among hematological disorders, and MiR 92a indicates colorectal cancer while controlling angiogenesis. The overview of miRNA signature as diagnostic, prognostic, therapeutic potential, and mechanism is summarized in Table 1.

Table 1: Overview of miRNA signature as diagnostic, prognostic, therapeutic potential and mechanism.

miRNA Signature	Diagnostic Potential	Change in miRNA Levels	Therapeutic Potential	Prognostic Potential	Mechanism	References
miRNA-21	High in certain cancers(e.g. lung)	Increased in cancers (e.g., lung cancer, breast cancer, colorectal cancer)	Potential target for cancer therapy	Indicator of poor prognosis in cancer	Regulates apoptosis and cell proliferation	[27]
miRNA-15	Indicators of inflammatory diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus) and inflammatory diseases (e.g., Crohn's disease, ulcerative colitis)	Increased in autoimmune (e.g., rheumatoid arthritis, systemic lupus erythematosus) and inflammatory diseases (e.g., Crohn's disease, ulcerative colitis)	Modulation in autoimmune disorders	Prognosis of chronic inflammation	Involved in immune response modulation	[28]
miRNA-34	Marker for neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease)	Decreased in neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease)	Role in Alzheimer's treatment	Predicts disease progression	Influences neuron survival and function	[29]
miRNA-122	Diagnostic for liver diseases (e.g., hepatitis C, liver cirrhosis, liver cancer)	Increased in liver diseases (e.g., hepatitis C, liver cirrhosis, liver cancer)	Therapeutic target in hepatitis C	Indicates liver fibrosis severity	Involved in cholesterol and lipid metabolism	[30]
miRNA-16	Biomarker for cardiovascular diseases (e.g., heart failure, myocardial infarction)	Decreased in cardiovascular diseases (e.g., heart failure, myocardial infarction)	Potential in heart failure treatment	Associated with cardiovascular risk	Modulates cardiac cell apoptosis	[31]

miRNA-200	Identified in various cancers (e.g., ovarian cancer, breast cancer)	Decreased in advanced cancers (e.g., ovarian cancer, breast cancer)	Role in preventing metastasis	Indicates cancer progression	Regulates epithelial-to-mesenchymal transition	[32]
miRNA-125b	Indicators for breast and ovarian cancers (e.g., breast cancer, ovarian cancer, prostate cancer)	Increased for breast and ovarian cancers	Target in certain cancer treatments	Predictive chemotherapy response	Modulates cancer cell proliferation	[33]
miRNA-let-7	Low in lung and breast cancers (e.g., lung cancer, breast cancer)	Low in lung and breast cancers	Target in lung and breast cancer therapy	Predicts survival in cancer patients	Regulates oncogenes and cell cycle	[34]
miRNA-223	Elevated in hematological disorders (e.g., acute myeloid leukemia, chronic lymphocytic leukemia)	Elevated in hematological disorders	Potential in blood disorder treatments	Prognostic in myeloid cancers	Involved in hematopoiesis and immune function	[35]
miRNA-92a	Indicator for colorectal cancer (e.g., gastric cancer, breast cancer)	Overexpressed in colorectal cancer	Role in angiogenesis inhibition in cancers	Associated with metastasis in cancers	Regulates angiogenesis and cell proliferation	[36]

Preanalytical Conditions for Circulating miRNAs

Circulating microRNAs (miRNAs) are small, non-coding RNAs that play critical roles in gene regulation and are stable in bodily fluids, making them promising biomarkers for various diseases. However, the accurate measurement of circulating miRNAs is heavily influenced by preanalytical conditions, which include factors from sample collection to RNA extraction. Proper handling of these steps is crucial for reliable results.

Sample Collection

RNase-free tubes designed for plasma or serum collection are recommended. Different blood collection tubes can affect miRNA yield and quality. Tubes containing EDTA, heparin, or citrate as anticoagulants can affect miRNA measurements differently. EDTA tubes are generally preferred as heparin can inhibit downstream PCR reactions, and citrate may affect miRNA quantification [37]. During blood draw or processing, hemolysis can release intracellular miRNAs, which may contaminate the sample and skew results [38,39]. Visual inspection of plasma/serum for pink discoloration and measuring hemolysis markers like miR-451a and miR-23a can help identify contaminated samples [40].

Sample Processing

To obtain plasma or serum, blood samples must be processed promptly. The standard protocol involves two-step centrifugation: an initial low-speed spin (e.g., 1,500–2,000 g) to separate plasma/serum and a second high-speed spin (e.g., 10,000 g) to remove cellular debris and platelets. Improper centrifugation can lead to contamination by cellular miRNAs. Delays in processing and incorrect storage temperatures can degrade miRNAs. It is crucial to keep samples on ice immediately after collection and to process them within two hours to prevent RNA degradation [41,42].

Storage Conditions

Plasma or serum samples should be stored at -80°C for long-term preservation of miRNAs. Repeated freeze-thaw cycles should be avoided as they can degrade miRNAs and alter expression profiles. Long-term storage stability can vary depending on the miRNA. Some studies suggest that miRNAs remain stable for months at -80°C, but it is recommended that samples be used as soon as possible after thawing [43].

RNA Extraction

The efficiency of miRNA extraction can vary significantly between commercially available kits. Kits with phenol-chloroform extraction steps are commonly used; however, automated systems are also available. Each method has its pros and cons regarding yield, purity, and consistency. Using exogenous spike-in controls (like synthetic miRNAs from other species) during extraction can help assess the efficiency and variability of the extraction process. Several column-based Kits are also used such as miRNeasy Mini Kit (Qiagen), and NucleoSpin miRNA Kit (Macherey-Nagel) and Magnetic Bead-based Kits also utilized such as Exosome Plus™ MicroRNA Isolation Kit (Thermo Fisher Scientific) [44,45,46].

Normalization Strategies

Accurate quantification requires appropriate normalization. Common strategies include the use of endogenous controls (such as miR-16), exogenous spike-in controls, or global mean normalization. However, no universal standard exists, making normalization a critical point of variability [47].

Small RNA library preparation kits for NGS

Small RNA library preparation kits are essential tools for next-generation sequencing (NGS) of circulating miRNAs. Examples include TruSeq Small RNA Library Prep Kit (Illumina) as it offers a streamlined workflow for efficient small RNA sequencing and NEBNext Small RNA Library Prep Kit (New England Biolabs) known for its high sensitivity and accuracy in capturing small RNA [48,49]. A brief overview of the preanalytical conditions for circulating miRNAs use in diagnostic is shown in Table 2.

Table 2: Overview of the preanalytical Conditions for Circulating miRNAs use in diagnostic.

S. No.	Category	Description	References
1.	**Sample Types**	**Blood samples:** EDTA, heparin, or citrate tubes.	[37]
2.	**Tube Recommendations**	RNase-free tubes and collection devices are essential. Tubes designed for plasma or serum collection are recommended.	[38,39]
3.	**Isolation Methodologies**	**Column-based Kits:** miRNeasy Mini Kit (Qiagen), NucleoSpin miRNA Kit (Macherey-Nagel).	[44]
		Magnetic Bead-based Kits: Exosome Plus™ MicroRNA Isolation Kit (Thermo Fisher Scientific).	[45]
		Phenol-Chloroform Extraction: The traditional method requires careful handling. (Invitrogen TM TRIzolTM Reagent and QIAzol Lysis Reagent).	[46]
4.	**Small RNA Library Prep Kits for NGS**	**TruSeq Small RNA Library Prep Kit (Illumina):** Streamlined workflow for small RNA sequencing.	[48]
		NEBNext Small RNA Library Prep Kit (New England Biolabs): High sensitivity and accuracy for small RNA capture.	[49]
5.	**Considerations**	Ensure compatibility between the library preparation kit and the RNA isolation method to maintain high-quality sequencing results.	[50]

Clinical Applications in Cancer Diagnosis

Circulating miRNAs (microRNAs) are being investigated as non-invasive blood biomarkers to assist with cancer diagnosis. Their non-invasive nature provides many advantages in clinical applications, including stable detection in blood and identification of cancer-specific miRNAs across many cancer types. MiRNAs have been associated with prognosis, survival, and drug resistance among cancer patients providing us with another tool for predicting treatment outcomes. However, challenges like poor diagnostic specificity, reproducibility, and individual factors influencing miRNA expression must be overcome to be effectively managed. Although miRNAs as biomarkers for cancer diagnosis remain challenging, researchers continue to actively explore their use as blood-based biomarkers through clinical trials that investigate therapy, diagnosis, and prognostication applications of miRNAs. MiRNAs offer promise as blood-based biomarkers for cancer diagnosis; however, more research and validation must be performed before being fully adopted as clinical tools. Therefore, miRNAs hold great promise as blood-based cancer biomarkers but require further development for full clinical implementation [21,51].

MiRNA expression profiles in tumor tissues provide invaluable insights for cancer diagnosis, serving as indicators to distinguish cancerous from noncancerous tissues. MicroRNAs have proved useful for cancer diagnosis in clinical applications, particularly with tissue-specific expression profiles. Genome-wide profiling has revealed that miRNA expression signatures correlate to tumor type, tumor grade, and clinical outcomes - making them promising biomarkers in cancer diagnosis and prognosis. However, identifying important miRNA targets in cancer and validating specific signatures as biomarkers remain key milestones in diagnostics [52]. Cancer cells contain different miRNA profiles that offer potential as diagnostic or prognostic biomarkers. The miRNAs have been associated with prognosis, survival, and drug resistance among cancer patients - making them useful tools in predicting outcomes of treatment. Unfortunately, however, challenges such as diagnostic specificity, reproducibility, and individual factors that influence miRNA expression must first be overcome to fully take advantage of miRNAs' clinical applications in cancer diagnosis and prediction [53,54].

Tissue-specific miRNA expression profiles in cancer diagnosis have shown potential in identifying cancer types, determining tumor stage, and predicting patient outcomes. Some examples of tissue-specific miRNA expression profiles in cancer diagnosis include:

A comprehensive profile of miRNAs in cancer has been established by analyzing miRNA expression in various cancer types, such as prostate, lung, stomach, pancreas, and thyroid. This study identified a "miRNoma" in cancer, consisting of overexpressed and downregulated miRNAs, some of which are well-characterized cancer-associated miRNAs like miR-17-5p, miR-20a, and miR-21 [55].

MicroRNAs (miRNAs) have shown potential as non-invasive diagnostic biomarkers in various diseases, including cancer. Some examples of miRNA biomarkers used in non-invasive diagnostics include Circulating miRNAs and Exosomal miRNAs. Circulating miRNAs are present in blood, such as serum or plasma, have been proposed as useful diagnostic biomarkers in cancer, as they can be measured in routine clinical diagnoses [56,57]. Exosomal miRNAs are small vesicles released by cells that can contain miRNAs. Exosomal miRNAs can be isolated from blood and have been suggested as potential non-invasive biomarkers for cancer [58].

Correlation between miRNA Expression and Cancer Prognosis

MicroRNAs play an essential part in cancer prognosis. Their expression levels correlate to clinical outcomes such as tumor stage, lymph node involvement, and overall survival rates. The miRNA profiles in cancerous and normal cells demonstrate promise as potential prognostic biomarkers; studies conducted on hepatocellular carcinoma found 414 gene-miRNA associations that provided strong prognostic information. Researchers discovered a correlation between miRNA expression levels and overall survival for various cancers such as HCC [59].

Identification of High-Risk and Low-Risk Patients

MicroRNAs have emerged as key prognostic indicators when diagnosing cancer patients and can prove particularly helpful when performing liquid biopsies. The miRNAs provide essential data regarding patients who are likely to survive long term, have a health-free prognosis, and respond well to treatments. Furthermore, miRNAs offer multiple advantages that include being less intrusive as well as higher precision. Bioinformatics development and diagnostic technology have greatly assisted with using miRNAs for cancer diagnosis and prognosis, with additional studies underway that may lead to their use within clinical settings [54,60]. MiR-210 and miR-141 prognostic miRNAs for breast cancer patients provide helpful prognostic information that enables physicians to distinguish those at increased risk for recurrence from those who stand a greater chance for survival, thus making more informed treatment choices as far as intensities or follow-up schedules are concerned [61].

Prognostic Value of Circulating miRNAs

Circulating miRNAs found in body fluids such as blood, saliva, and urine have emerged as non-invasive prognostic biomarkers due to their stable and accessible nature. Studies have identified specific miRNA signatures found circulating that correlate with clinical outcomes, such as miR-155 and 21 which have been linked with reduced survival for various cancer types. The miRNA detection and analysis offer promise as a non-invasive means to enhance prognostic assessments and track disease progression without using invasive procedures, yet research into miRNA is in its infancy with results often lacking reproducibility. Standardized protocols for sample collection, transport,

storage, and data analysis can help researchers overcome any differences among research teams. Although miRNAs do not meet all criteria to act as definitive prognostic biomarkers for cancer patients, circulating miRNAs have shown promise as potential clinical biomarkers and more research and standard protocols need to be put in place to fully establish them as useful prognostic indicators for this purpose [62].

Integration with Traditional Prognostic Factors

MicroRNAs have shown enormous promise as prognostic biomarkers of cancer, and, when used alongside more established factors, can significantly enhance their predictive power. Their role can be understood from two angles. First, they act as potential precursors and second as potential biomarkers of future outcomes of treatment plans.

MicroRNAs provide invaluable insight into cancer biology and risk stratification, linking with specific molecular pathways or tumor characteristics to create more precise prediction models of overall or disease-free survival [63]. Integration also facilitates the identification of subclasses that exhibit different clinical effects or therapeutic responses for more tailored plans that provide superior patient results. An analysis that integrates miRNA data with traditional prognostic factors can identify miRNA signatures associated with specific clinical outcomes, including overall survival, disease-free survival, and treatment response [64]. Integrating miRNA expression data with traditional clinicopathological factors can significantly enhance the accuracy of prognostic prediction models. Algorithms incorporating multiple miRNAs have shown superior prognostic performance compared to using only traditional factors alone; such integrated models could give a more complete understanding of disease behavior as well as aid in individualizing treatment plans. Furthermore, this integration will establish miRNA's clinical utility in prognostication [65].

Therapeutic Implications of MicroRNAs in Cancer

MicroRNAs (miRNAs) have garnered significant attention as potential therapeutic targets in cancer treatment. Their dysregulated expression in cancer cells, particularly oncogenic miRNAs, provides an opportunity for therapeutic intervention. Here, we explore two strategies for targeting oncogenic miRNAs: using antisense oligonucleotides and small molecule inhibitors.

Antisense Oligonucleotides

Antisense Oligonucleotides (ASOs) are synthetic sequences of nucleotides designed to specifically bind with and interfere with target microRNAs (miRNAs) to inhibit their function and potentially restore expression of tumor suppressor genes. ASOs function by base-pairing their oncogenic miRNA targets and disturbing miRNA binding sites on messenger RNA (mRNA) for cancer treatment. This disrupts its downstream regulatory effects while potentially restoring expression of tumor suppressor genes; ASOs can also be chemically modified further to enhance stability and specificity for better performance results as preclinical studies have demonstrated inhibition of tumor growth along with increased chemotherapy response/sensitivity/sensitivity for various cancer types.

ASOs have the potential to dramatically decrease the activity of oncogenic miRNAs like miRNA-23a and miRNA-106b that play key roles in tumorigenesis, by targeting them directly [66]. Their specificity to target miRNAs and stability engineering ensures effective binding/inhibition; such qualities make ASOs highly sought-after therapeutic agents for cancer treatments [67].

ASOs may cause fewer off-target effects compared to traditional small interfering RNAs (siRNAs) since they target specific miRNAs rather than specific mRNAs; this increased accuracy helps decrease side effects while improving therapeutic results [38]. Chemical modifications of ASOs have enhanced their stability for effective binding to target miRNAs and inhibiting their function (68). ASOs can be modified using cell-penetrating peptides or other delivery systems to enhance uptake by cancer cells for increased therapeutic efficacy; this improved drug delivery can result in more successful cancer therapy [69].

Antisense Oligonucleotides (ASOs) targeting miRNAs have been developed as anticancer treatments, including anti-miRNA oligonucleotides (AMOs). AMOs specifically target specific miRNAs like miRNA-23a and miRNA-106b which tend to become upregulated during cancer development. An AMO can inhibit tumorigenesis by decreasing expression levels of target miRNAs (60). AntagomiRs are another miRNA-targeting oligonucleotide that works by binding and blocking specific miRNAs overexpressed in cancerous cells, acting like anticancer drugs by binding to and silencing their activity [70]. AntagomiRs are synthetic double-stranded oligonucleotides designed to overexpress miRNA targets that in turn regulate cancer cell proliferation; miRNA mimetics could potentially act as cancer therapeutic agents [71].

There have been various clinical trials using antisense oligonucleotides (ASOs) to target miRNAs for cancer therapy. One such ASO, called MRX34 (liposomal formulation of miR-34a mimic), was recently evaluated in a phase I trial as a treatment of advanced solid tumors. Trial data demonstrated that MRX34 was well tolerated and showed promising antitumor activity [72]. Meanwhile, ASOs such as AZD9150 targeting miR-221 which is overexpressed in various cancers tested in phase I clinical trials for treating advanced solid tumors with positive results [73]. RG-012, an ASO that targets miR-21 overexpression found in various cancers, was tested in a phase I clinical trial for advanced solid tumors with promising results [74].

Small Molecule Inhibitors

Small molecule inhibitors possess ways of targeting oncogenic miRNAs in cancer. Small molecule inhibitor molecules interact with specific miRNAs or components of their biogenesis pathway to inhibit processing or function, binding directly

with target mRNA or disrupting key proteins involved in the biogenesis of miRNA. By blocking oncogenic miRNA activity these inhibitors aim to restore normal gene expression patterns and restrain tumor growth [57]. Several small molecule inhibitors targeting oncogenic miRNAs are currently undergoing preclinical and clinical evaluation, showing their therapeutic potential [75].

Small molecule inhibitors target oncogenic miRNAs through multiple mechanisms, including inhibiting transcription or interfering with loading onto an RNA-induced silencing complex (RISC). For instance, one small molecule inhibitor was discovered that was effective at targeting miR-21 transcription, effectively decreasing its expression. Furthermore, small molecules can interfere with loading miRNA onto AGO2, an essential step for its functionality; such antagonizers may be identified through high-throughput screening, providing another effective approach in targeting oncogenic pathways while potentially modulating miRNA activity or even developing therapeutics targeted specifically against miRNA [76].

Small molecule inhibitors can easily be structurally modified to increase selectivity, stability, and bioavailability for use as therapeutic agents, leading to their rapid development. They have proven particularly promising as tools in miRNA therapeutic development due to their bioactivity and wide chemical space [77]. Furthermore, small molecule inhibitors may be combined with chemotherapy or radiation therapies to further boost efficacy while decreasing side effects [78].

Small molecule inhibitors of miRNA have been discovered for use in cancer therapy. One such inhibitor specifically targeted miR-21 transcription and reduced its expression levels; further, this was proven effective against oncogenic pathways [79]. Studies on small molecule inhibitors that modulate RNAi pathways could potentially recover tumor-suppressor miRNAs while simultaneously decreasing the expression and function of oncogenic genes [77]. Enoxacin is a small molecule that serves as a cancer-specific growth inhibitor by increasing TAR RNA-binding protein 2-mediated microRNA processing [53].

Restoration of Tumour -Suppressive miRNAs

Tumor-suppressive miRNAs are crucial in regulating the expression of genes associated with tumor growth and progression. By restoring their levels and activity, it is possible to regain control over dysregulated gene expression contributing to cancer development. This targeted approach allows for precise modulation of specific genes and pathways involved in tumorigenesis. Tumor-suppressive miRNAs typically target multiple oncogenes or genes involved in tumor-promoting pathways, enhancing the overall therapeutic effect. Restoration of tumor-suppressive miRNAs holds great therapeutic potential in cancer treatment. The two primary strategies used for this purpose are the use of miRNA mimics and viral vectors for miRNA delivery [80].

Many applications of tumor-suppressive miRNA restoration have shown promise in preclinical research studies. Tumor-suppressive miRNAs have been discovered to effectively limit tumor growth across several cancer types. Restoration of miR-143 and miR-145 for colorectal cancer was seen to significantly reduce tumor size and growth rates [71]. Restoring miR-26a to HCC patients showed a significant reduction in tumor growth. Tumor-suppressive miRNAs have long been established as effective ways of fighting cancer cell proliferation.

The miRNA mimics are synthetic double-stranded RNA molecules created to mimic the function of natural microRNAs found within our bodies (miRNAs). The miRNA mimics are composed of two components; an “miR-mimicking guide strand” designed to closely resemble mature miRs; and an accompanying passenger strand. miR-Mims can then be added into an RNA-Induced Silencing Complex (RISC), where their guide strands bind directly with specific target mRp for degradation or inhibition of translation into proteins for suppression through degradation or inhibition. miR-Mims can then be added into an RNA-Induced Silencing Complex (RISC), where their guide strands bind directly with specific target mRp for degradation or inhibition of translation into proteins for suppression through degradation or inhibition. The suppression of repressor gene expression through direct binding to the specific target mRMP targets stops the degradation or translation can also be achieved, by utilizing the replicators of miRmims-mims replicators as potent inhibitors.

Normal cells use these microRNAs as tumor suppressors by using them to modulate genes responsible for proliferation, differentiation, and death in their cells. However, tumor cells often alter or block certain miRNAs that regulate processes governing development resulting in unchecked tumor expansion and growth. The miRNA mimics possess the capability to replace dysregulated immune-suppressing miRNAs that restrict tumor growth with miRNAs that restore the normal functions that were absent previously due to deregulation. They do this by mimicking certain immune-suppressing miRNAs known as immuno-suppressive miRNAs that suppress tumor cells; for cancer cells, this restores beneficial miRNA functions that previously weren’t functioning normally or were missing altogether. The miRNA mimics can use their ability to restore miRNA function to combat tumors by stopping oncogene expression (cancer-promoting genes), metastasis-promoting genes, and resistance-inducing genes from manifesting. Their benefits in fighting chemotherapy resistance and metastasis result in increasing Apoptosis rates which inhibit proliferation while slowing cancer cell metastatic spread, with lower invasion risks overall because of their use.

The miRNA mimics represent advancements in cancer therapy due to their unique capability of simultaneously targeting several genes or pathways. several approaches for combating cancer offer numerous advantages, especially as they may help limit resistance development associated with traditional approaches. The miRNA mimics the role of naturally occurring tumor suppressor miRNAs to offer more effective, targeted therapies than chemotherapy treatments. Reversing epigenetic change

and reinstating gene expression that suppresses tumors adds significantly to their value as therapeutics and cancer treatments. The miRNA mimics offer customized treatments for cancer based on each person's genetic makeup.

Viral vectors are being explored as a potential delivery system for tumor-suppressive miRNAs. These vectors, which can be packaged into viral particles like lentiviruses or adenoviruses, can deliver and express specific miRNAs in cancer cells. Once introduced, these vectors facilitate the intracellular production of tumor-suppressive miRNAs, restoring their normal function. This approach has shown promise in preclinical studies, demonstrating tumor growth inhibition and increased sensitivity to cancer treatments. These strategies aim to counteract tumorigenic effects and restore normal gene regulation. However, further research is needed to optimize delivery methods, enhance therapeutic efficacy, and ensure the safety of these interventions for clinical applications [81]. Further research is needed to optimize delivery methods, enhance therapeutic efficacy, and ensure the safety of these interventions for clinical applications. Engineers modify viral vectors like lentiviruses, adenoviruses, or AAVs to create a safe and efficient delivery system. These modifications remove viral genes essential for replication and pathogenicity, leaving behind necessary components for viral entry and cargo delivery [82]. Therapeutic miRNA sequences are introduced into a viral vector as transgenes, either as synthetic mimics or precursor sequences that undergo processing in target cells to yield functional miRNA [83]. Modified viral vectors bind to specific cell surface receptors through viral envelope proteins, allowing them to enter target cells via receptor-mediated endocytosis or membrane fusion [84]. Once inside, the vector uncoats, releasing the therapeutic miRNA cargo into the cytoplasm. Viral vectors like lentiviruses and adenoviruses release their genetic material into the nucleus [85]. MiRNA transgenes are delivered into the cellular miRNA processing pathway, where they undergo further processing to generate mature miRNAs. These mature miRNAs bind to target mRNAs, leading to gene silencing or translational repression. This results in the restoration or modulation of specific gene expression patterns affected by dysregulated miRNAs in target cells [86].

Studies have explored the use of viral vectors as delivery vehicles in miRNA-based therapy. One of the studies found that lentiviral vectors can deliver tumor-suppressive miRNA-145 to colon cancer cells, inhibiting cell growth, apoptosis, and tumor formation in animal models. This highlights the potential of lentiviral vectors in cancer therapy, as they are downregulated in many cancers [87]. Another study found that lentiviral vectors can deliver synthetic miR-34a mimics, a tumor-suppressive miRNA, to glioblastoma multiforme (GBM). The delivery suppressed glioma cell proliferation, induced apoptosis, and inhibited tumor growth in xenograft models, demonstrating the therapeutic potential of miR-34a mimics in GBM treatment [88]. Viral vectors, including adeno-associated viruses (AAVs) and lentiviruses, have shown significant potential in various biomedical applications. They can efficiently deliver therapeutic miRNAs to target cells and tissues, allowing for gene expression modulation and disease-causing genetic abnormalities correction. They have shown promise in cancer therapy by targeting tumor-suppressive miRNAs or miRNA-based therapeutics, increasing therapeutic efficacy while minimizing off-target effects [89].

Challenges and Future Perspectives in miRNA Therapeutics

MiRNA therapeutics provide great promise; however, numerous key obstacles must first be cleared away to enable successful implementation to take place. Understanding and overcoming challenges is crucial to the development of miRNA treatments and advancing towards their clinical use. The main challenge in miRNA research is developing efficient delivery mechanisms that can target specific tissues or cells to deliver miRNA. Ergonomically targeting miRNA therapeutics without off-target effects is of vital importance and requires extensive research to guarantee the delivery of therapeutics that precisely target their intended genes without unintendedly altering the expression of other non-target ones, thus minimizing side effects and potential side effects.

Understanding the pharmacokinetics and pharmacodynamics of miRNA therapeutics is integral to optimizing dosage, frequency of administration, and duration of therapy. Strategies designed to increase stability, half-life, tissue accumulation, and immunogenicity must also be thoroughly explored as part of any miRNA therapeutics' clinical translation plan. Current research endeavors focus on optimizing miRNA molecules, delivery systems, and long-term safety profiles to enable reliable clinical use. Utilizing miRNA biomarkers and their functional significance across diseases will enable clinicians to more effectively stratify patients and create tailored treatment approaches. Advancements in high-throughput sequencing technology, bioinformatics, and data integration methods will aid researchers in discovering novel miRNA biomarkers and designing tailored therapies using miRNA-based therapeutic approaches. MiRNA therapeutics pose complex regulatory requirements and hurdles that must be carefully considered before entering clinical development. Researchers, clinicians and regulatory authorities working in harmony to develop stringent protocols and guidelines can ensure safe efficacy [90,91].

Prospects involve continued studies into miRNA biology, the development of better delivery systems, and adapting current technological approaches to meet future challenges. Combinatorial approaches may increase therapeutic efficacy when miRNA therapies are combined with chemotherapy or immunotherapy treatment modalities like chemotherapy. Many studies are being undertaken to demonstrate the value of miRNAs as diagnostic markers. Davey MG conducted a detailed investigation analyzing miRNA expression patterns in blood samples from lung cancer patients and healthy controls and discovered specific miRNAs were significantly upregulated among cancer patients (miR-21 and miR-486 specifically), showing their potential as non-invasive diagnostic markers [92]. One case study provided compelling evidence for

miRNA's usefulness in prostate cancer diagnosis and prognosis. Researchers identified a signature of miRNAs that accurately discriminated between prostate cancer and benign prostatic hyperplasia (BPH) using miRNA expression profiling; specific miRNAs like miR-21 and 221 were linked with aggressive prostate cancers with poor prognoses [93].

Clinical case studies and success stories demonstrating the potential of miRNA-based therapies

Liposomal formulation of synthetic miR-34a mimic MRX34 was subjected to an initial clinical trial for treating advanced solid tumors like melanoma and lung cancer in phase one clinical studies. Therapy using MRG-106, a synthetic oligonucleotide inhibitor targeting miR-155, demonstrated promising antitumor activity with partial responses and disease stabilization reported from several patients [71]. MRG-106 was tested in a phase I clinical trial to treat CTCL lymphomas; its effectiveness against them proved promising. Treatment resulted in significant clinical responses, including reductions in tumor size and improvement of skin-related symptoms [94]. RG-012, an anti-miR-21 oligonucleotide, was tested as part of a phase I/II clinical trial for Alport syndrome - a genetic kidney condition - where preliminary results suggested target engagement as well as potential therapeutic advantages; suggesting miRNA therapies might hold great promise [95].

These examples highlight the clinical translation of miRNA-based therapies across diverse disease settings. They demonstrate the potential of miRNA modulation to target specific disease mechanisms and offer new therapeutic strategies.

Miravirsen's clinical trial with patients living with Hepatitis C virus infection showed substantial reductions in HCV RNA levels, sustained viral response rates among some patients, and improved liver function and reduced liver fibrosis; thus, demonstrating the power of miRNA therapies for improving HCV infection outcomes [96]. Another report demonstrated the efficacy of miR-34a mimic therapy given to a pediatric neuroblastoma patient; treatment led to tumor regression and complete remission - further showing their utility in personalized medicine and treating aggressive cancers [97]. Clinical results from an anti-miRNA oligonucleotide trial targeting miR-155 were promising; some participants with cutaneous T-cell lymphoma (CTCL) experienced complete responses accompanied by improved symptoms, reduced tumor burden, and extended progression-free survival. These data underscore the potential role played by miRNA therapies in improving patient outcomes related to hematological malignancies [98].

An analysis conducted retrospectively indicated that high miR-34a expression in lung tumor tissues from patients diagnosed with non-small cell lung cancer (NSCLC) is linked with improved prognosis. Researchers discovered that patients expressing high levels of miR-34a had significantly greater overall and progression-free survival compared with those displaying lower miRNA expression, further supporting its prognostic value and role as an aid for treatment decisions and patient management. These clinical case studies and success stories showcase how miRNA therapies have made positive impacts on various diseases [93]. The miRNA modulation shows great promise in terms of improving treatment responses, prolonging survival rates, inducing remission, and alleviating symptoms associated with cancers or viral infections.

Future Directions

The miRNA-based therapies combined with existing treatments such as chemotherapy, immunotherapy, or targeted therapies have the power to significantly enhance patient outcomes. However further research must be conducted to identify and validate miRNA biomarkers that will enable accurate diagnosis, prognosis, and response prediction from targeted therapy treatments such as chemotherapy or immunotherapy - helping guide treatment decisions more specifically and improve management for each patient. Innovative delivery systems including exosomes, peptide-based carriers, and genome editing tools have recently been created to boost miRNA therapeutic efficiency while clinical trials with diverse patient populations are crucial to establish clinical efficacy, safety, and long-term benefits associated with miRNA therapies [99].

Emerging Technologies for miRNA Profiling

MicroRNA (miRNA) profiling is crucial for understanding biological processes and diseases. Advanced technologies like Next-Generation Sequencing (NGS) and Digital PCR (dPCR) offer unique capabilities and applications in miRNA profiling. NGS is used in cancer research for profiling and quantifying microRNAs, including their expression levels. dPCR partitions samples into thousands of individual reactions, making it suitable for measuring miRNA expression levels across biological samples. Single-cell sequencing technologies like scRNA-seq have revolutionized miRNA profiling by allowing analysis at the single-cell level, offering insight into miRNA expression heterogeneity and dynamics. Nanostring nCounter uses hybridization-based detection and quantification technology to enable simultaneous identification and quantification of multiple miRNAs within one assay with high sensitivity. Droplet Digital PCR (ddPCR) is accurate and sensitive for quantifying miRNAs found in samples. Microarrays were once widely used for miRNA expression profiling studies, but their use has declined due to NGS technologies. Small RNA-Seq targets small RNA molecules like miRNAs, allowing for the identification of known and novel miRNAs and their expression patterns. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) is the go-to technique for miRNA analysis due to improved sensitivity, specificity, and accuracy. Locked Nucleic Acid (LNA) technology enhances probe binding affinity to miRNAs, increasing sensitivity and specificity. Bead-based miRNA profiling assays use bead arrays containing probes specific for miRNA to quantitatively examine expression levels of multiple miRNAs. Mass spectrometry techniques like MALDI-MS and SRM can be used for miRNA profiling. In Table 3. we summarise the technique with its key features, outcome, and significance.

Table 3: Emerging techniques with their key features outcome and significance.

Technique	Key Features	Outcome and Significance	References
Next Generation Sequencing	Comprehensive profiling of miRNAs	NGS enables the identification of dysregulated miRNAs, offering potential non-invasive biomarkers for early lung cancer detection.	[73]
Digital PCR	Absolute quantification of miRNAs	dPCR-based miRNA profiling holds potential for breast cancer diagnosis and personalized medicine approaches.	[100]
Single Cell Sequencing	Analysis of miRNA expression at the single-cell level	Single-cell miRNA profiling provides insights into miRNA expression heterogeneity during neuronal development.	[101]
Nanostring nCounter Technology	Simultaneous detection and quantification of multiple miRNAs in a single assay	Nanostring nCounter technology enables the identification of miRNA biomarkers for bladder cancer diagnosis and prognosis.	[102]
Droplet Digital PCR	Absolute quantification of miRNAs with high precision and sensitivity	Identified a miRNA-based signature for ddPCR-based miRNA profiling shows potential as a non-invasive diagnostic tool for colorectal cancer.	[103]
Microarrays	Simultaneous detection and quantification of multiple miRNAs	Microarray profiling identified miRNAs involved in myocardial infarction, providing insights into cardiac disease mechanisms.	[104]
Small RNAseq	Sequencing of small RNA molecules, including miRNAs	Small RNA-Seq enables the identification of miRNAs associated with pregnancy-related complications.	[105]
qRTPCR	Widely used technique for miRNA expression analysis	qRTPCR-based miRNA profiling provides insights into the involvement of dysregulated miRNAs in Alzheimer's disease	[106]
LNA-based miRNA profiling.	Enhanced probe binding affinity using Locked Nucleic Acid (LNA) technology	LNA-based miRNA profiling allows for the identification of region-specific miRNA expression patterns in the bra in.	[107]
Bead-based miRNA profiling	Quantitative analysis of miRNA expression levels using bead arrays	Bead-based miRNA profiling enables the identification of miRNA signatures with potential clinical implications in hepatocellular carcinoma.	[108]
Mass Spectrometry	Mass spectrometry-based methods for miRNA profiling	Mass spectrometry-based miRNA profiling offers a potential avenue for identifying miRNA biomarkers in prostate cancer.	[109]
Functional miRNA profiling	Study of miRNA activity and their effects on target genes	Functional miRNA profiling provides insights into the regulatory functions of specific miRNAs in gene expression and cancer biology.	[110]

Bioinformatics Tools for miRNA Data Analysis

Bioinformatics tools like miRDeep2 and miRExpress are crucial in miRNA research, identifying and quantifying miRNAs through quality control, alignment to known databases, and prediction of novel miRNAs. This comprehensive process helps understand miRNA expression patterns in biological samples and diseases. MiRNA target prediction is crucial for understanding miRNA function. Tools like TargetScan and miRanda use algorithms to predict potential mRNA targets, focusing on sequence complementarity and evolutionary conservation. These tools help researchers infer miRNA regulatory roles in gene expression, enabling a better understanding of biological processes and disease mechanisms. DIANA-TarBase and miRTarBase are databases that aid in investigating and constructing regulatory networks involving miRNA-mRNA interactions, providing access to experimentally validated interactions, and enhancing understanding of miRNA roles across biological processes and diseases. DIANA-mirPath and miRWalk tool functional enrichment analyses give valuable insight into biological implications associated with miRNAs;

mapping miRNAs onto various biological processes provides useful evidence regarding regulatory roles related to cell cycle regulation, apoptosis regulation, and differentiation regulation activities as well as cell death regulation activities.

Differential expression analysis is crucial in miRNA research, revealing upregulated or downregulated miRNAs. Tools like edgeR and DESeq compare expression levels across conditions, enhancing understanding of miRNA regulation in various biological contexts.

Data integration and visualization are crucial in miRNA research, with tools like Cytoscape and miRBase serving as essential sources. Cytoscape provides an integrative genomic data portal, offering access to genomic information such as miRNA sequence, gene predictions, protein interaction networks, and other biological features. miRBase offers authoritative databases with miRNA sequences annotated with target sequence information, allowing for accurate analysis and interpretation. Cancerous cancer researchers use an integrated approach using bioinformatics tools to understand how miRNAs may play a part in disease. RNA-Seq data from cancerous and normal tissues is processed using miRDeep2, EdgeR, TargetScan, and DIANA-mirPath. These tools detect and quantify miRNAs, identify miRNAs with varying expression levels, and perform pathway analysis to reveal biological implications. Cytoscape visualizes complex miRNA-target interaction networks within cancer pathways, providing therapeutic targets. This innovative methodology identifies key miRNAs used and reveals functional roles within cancer pathways, revealing possible therapeutic targets. These bioinformatics tools are essential for miRNA research specifically related to cancer studies as in detail it is summarized in Table 4.

Table 4: Overview of Bioinformatics Tools and Processes in miRNA Research and Analysis.

Aspect	Tools	Tools	References
Identification and Quantification of miRNAs	miRDeep2, miRExpress	miRDeep2, miRExpress	[111]
miRNA Target Prediction	TargetScan, miRanda	TargetScan, miRanda	[112]
miRNA-mRNA Interaction and Network Analysis	DIANA-TarBase, miRTarBase	DIANA-TarBase, miRTarBase	[113]
Functional Enrichment Analysis	DIANA-mirPath, miRWalk	DIANA-mirPath, miRWalk	[114]
Differential Expression Analysis	edgeR, DESeq	Utilizing statistical models, identify miRNAs with significant expression changes across various conditions.	[115]
Data Integration and Visualization	Cytoscape, miRBase	Integrate and visualize miRNA, mRNA, and protein interaction data to gain greater insight into complex regulatory networks.	[116]
Example of Usage in Cancer Research	miRDeep2, edgeR, TargetScan, DIANA-mirPath, Cytoscape	Gather RNA-Seq data from cancerous/normal tissues; identify and quantify miRNAs; compare expression levels among gene targets; analyze differential expression and target gene targeting patterns, as well as visualize interaction networks.	[117]

Ethical Considerations and Challenges in miRNA Diagnostics

The miRNA diagnostics, which analyze genetic material, raise concerns about patient privacy and security. Safeguards should be in place to protect confidentiality and prevent unauthorized access. Informed consent is crucial, as patients should be fully informed about the purpose, benefits, limitations, and potential risks of testing. Proper genetic counseling should be offered to help patients understand the implications of test results and make informed decisions about medical interventions or family planning. Psychological implications of miRNA diagnostics, particularly if they reveal increased disease risks or genetic predispositions, should be addressed. Access to appropriate psychological support and counseling services is essential. Ethical considerations include ensuring clinical validity and utility of miRNA tests, which require rigorous validation studies. Prioritizing patient autonomy, privacy, data protection, informed consent, and equitable access can ensure the responsible and beneficial use of miRNA diagnostics in clinical practice [118].

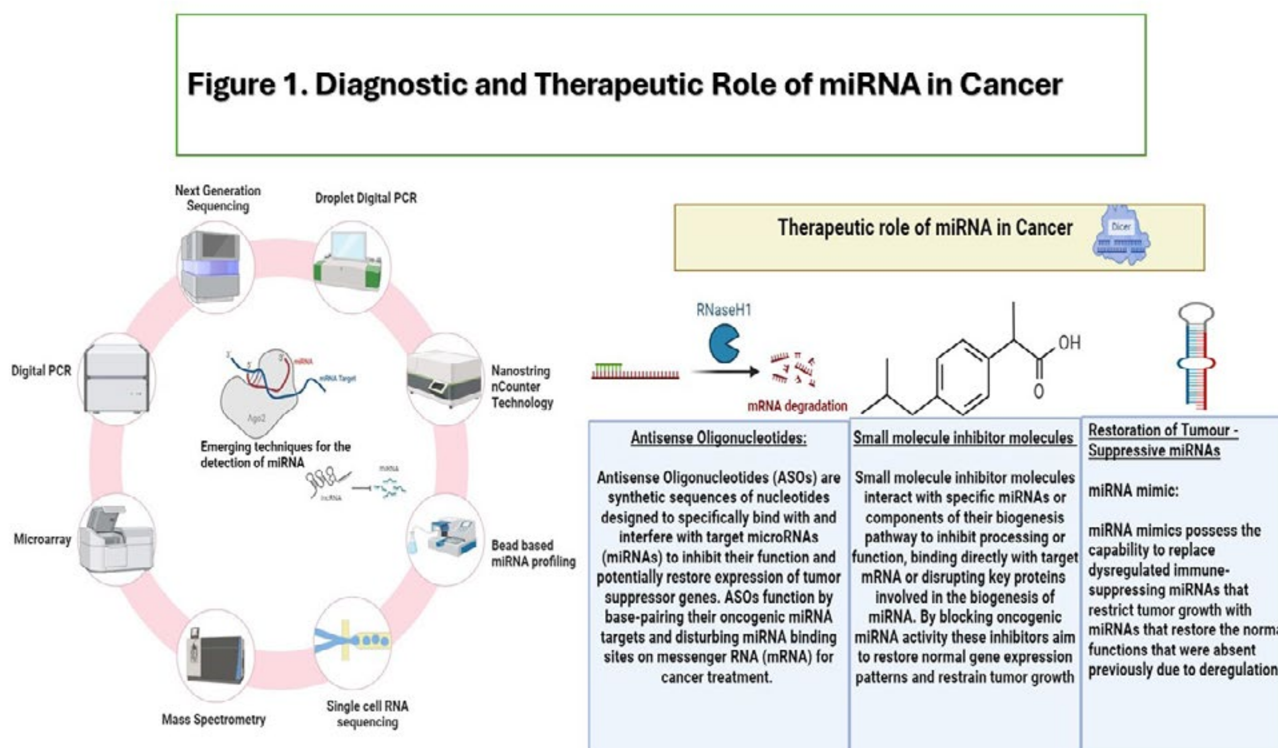
The miRNA mimic therapy is a novel cancer treatment approach that requires patients to be informed about its workings, benefits, risks, and experimental nature. Informed consent is crucial for patients to make informed decisions, which may require additional resources or consultations with healthcare professionals. Patients should be informed about alternative treatments and their right to withdraw at any point without affecting future care. Communication should be in an easily understandable language, avoiding technical jargon and using visual aids or metaphors. Understanding the patient's cultural background and beliefs is essential for their perception of advanced genetic therapies and treatment decisions. Discussions should also address the potential psychological impact of the treatment, including access to psychological support. Effective communication is an ongoing process, with regular updates on treatment progress, new findings, and concerns [119]. The development of miRNA therapeutics, particularly for cancer treatment, is a complex field with regulatory and ethical challenges. The approval process involves extensive preclinical and clinical trials, requiring clear guidelines for standardization and quality control. Ethical considerations include informed consent, patient understanding, data privacy, and potential long-term effects. Intellectual property rights and high costs could hinder research and development. Balancing risks and benefits, maintaining public trust, and fostering innovation are crucial for miRNA therapy advancement. A dynamic approach is needed to ensure patient safety and equity [120].

Conclusion

This review has examined the prominent function of microRNAs (miRNAs) in cancer research and their potential in diverse biological applications. The miRNAs, being non-coding RNA molecules, play a vital role in regulating gene expression by finely adjusting the amounts of gene expression through their interaction with messenger RNA (mRNA) molecules. Altered expression of miRNAs has been detected in various cancer types, indicating their role in the onset and advancement of cancer. Dysregulated miRNAs can serve as tumor suppressors by suppressing the production of oncogenes, or as oncogenes themselves by stimulating tumor development and metastasis. Furthermore, the disruption of miRNAs can affect crucial cellular pathways implicated in the progression of cancer, including as cell growth, programmed cell death, and the formation of new blood vessels.

Significantly, miRNAs have shown promise as significant diagnostic and prognostic indicators for cancer. Distinct miRNA expression profiles have been discovered that facilitate the distinction between healthy and malignant tissues. The differential expression patterns can be identified in diverse clinical samples, including tissue biopsies or liquid biopsies, providing a non-invasive method for early cancer identification [23]. Moreover, some miRNAs have demonstrated potential as prognostic markers, enabling the anticipation of patient outcomes and their reaction to therapies. To establish distinct miRNA signatures for different forms of cancer, it is necessary to conduct additional validation studies, considering the variability of tumors and patient groups. Conducting extensive clinical studies is necessary to validate the clinical usefulness of miRNAs as biomarkers for cancer diagnosis, prognosis, and therapy response as it is shown in Figure 1.

Figure 1: Diagnostic and Therapeutic Role of miRNA in Cancer.



Furthermore, continuous research endeavours are focused on creating novel therapeutic approaches that utilize the abnormal expression of miRNAs in cancer cells, hence creating opportunities for precise and targeted treatments. It is imperative to thoroughly examine the ethical implications and challenges associated with miRNA diagnostics and treatments. Essential factors to address include obtaining informed permission, effective patient communication, ensuring data privacy, and providing fair access to miRNA testing and therapy. The presence of regulatory systems and intellectual property rights will create further difficulties, requiring endeavors to guarantee thorough validation, standardization, and accessibility of miRNA-based therapeutics. Despite the difficulties encountered, the knowledge acquired from miRNA research holds significant ramifications for personalized medicine, illness detection, prediction, and therapy. miRNAs possess the capacity to transform cancer management by acting as crucial regulators and biomarkers in cancer research. Gaining insight into the distinct functions of miRNAs in various cancer types has the potential to enhance diagnostic methods, facilitate the development of tailored treatment strategies, and ultimately improve patient outcomes. Further examination and verification of miRNAs in cancer research will aid in the progress of precision medicine and the creation of innovative treatments.

Declaration of Conflict of interests

The authors of this article declare that there is no conflict of interest with regard to the content of this manuscript.

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Research Article

Diagnostic Accuracy of Creatinine-Based Equations for eGFR Estimation in Pakistanis: Evaluation of the European Kidney Function Consortium Equation vs the CKD-EPI Pakistan Equation

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Keywords
Renal function; equations; eGFR; Pakistani

Abstract

Introduction
Chronic Kidney Disease (CKD) is prevalent in Pakistan, necessitating accurate diagnostic methods. This study evaluates the CKD-EPI 2009, CKD-EPI 2021, CKD-EPI Pak, MDRD, and EKFC equations against creatinine clearance (CrCl) to determine their diagnostic accuracy for CKD in the Pakistani population.

Methods
n a retrospective cross-sectional study, data from 2,310 participants aged 18-70 were analyzed at The Aga Khan University in Karachi. Serum creatinine (SCr) and CrCl were recorded, and eGFR was calculated using five equations. Statistical analyses compared eGFR equations with CrCl, assessing sensitivity, specificity, and predictive values.

Results
EPI-Pak exhibited the highest sensitivity (95.15%) and agreement (94.85%) followed by EPI-2009 and EPI-2021 which showed the closest agreement with CrCl. Bland-Altman plots also indicated that EPI-Pak had the best agreement with CrCl.
Discussion
EPI-Pak outperformed other equations in estimating eGFR for the Pakistani population, aligning with previous recommendations for South Asians. EKFC, although highly specific, was less effective overall.

Conclusion
EPI-Pak is the most accurate equation for diagnosing CKD in the Pakistani population. Its clinical implementation could improve CKD diagnosis and patient outcomes. Future studies should further validate these findings with larger, diverse samples.

Introduction

Chronic Kidney Disease (CKD) is a global health issue, leading to significant morbidity and mortality. The prevalence of CKD in Pakistan lies between 12.5% to 31.2% [1]. CKD progression eventually leads to kidney failure, resulting in the need for renal replacement therapy either by dialysis or by renal transplantation [2]. The most common etiologies for CKD, Diabetes Mellitus and Hypertension, are also the most common comorbidities in Pakistan. Moreover, CKD is also a risk factor for cardiovascular complications, thus highlighting the need for the early diagnosis and management of CKD [3].

Renal function can be assessed by estimating the glomerular filtration rate (GFR) using values of serum creatinine and 24-hour creatinine clearance (CrCl). Estimated GFR (eGFR) based upon serum creatinine is considered to be the most reliable indicator of kidney function. Serum creatinine (SCr) based eGFR is calculated using various equations which have been derived and validated in various populations [4]. The Modification of Diet in Renal Disease (MDRD) equation utilizes an individual's SCr, whereas the Cockcroft-Gault (CG) formula uses an individual's CrCl adjusted for the body surface area and weight although it is taken on average. This assumption limits the CG formula when applied to overweight individuals. The MDRD equation surpasses the CG although it still has its limitations due to significant bias and may not be optimum in mild CKD cases [5, 6]. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) created an equation (CKD-EPI 2009) in 2009 for eGFR. This outperformed MDRD because of lesser bias, better precision as well as greater accuracy. However, since participants of racial and ethnic minorities were very limited in the study, the population it takes into account is relatively limited [7]. Because of this restriction, in 2015, CKD-EPI Pakistan was made by making some alterations to the factors in CKD-EPI equation which allowed CKD-EPI Pakistan to factor in the South Asian demographics as well [8]. However, after using inulin clearance as a gold standard to see competence of CKD-EPI Pakistan in comparison to MDRD, CKD-EPI Pakistan came out to be more reliable when applied to the Pakistani population. In 2021, the coefficient for race was removed from the CKD-EPI eGFR equation and the modified version of that equation was accepted by the National Kidney Foundation and the American Society of Nephrology, although upon comparison, the difference in values of eGFR calculated by both equations was quite minor [9].

Parallel to this, another equation was developed by the European Kidney Function Consortium (EKFC) in 2021. This equation surpasses the limitations of its predecessors while also given to laboratories to be incorporated without needing changes which makes it more convenient to use [10]. Given the limitations of existing GFR estimation equations in accurately diagnosing CKD in diverse populations, there is a need to evaluate their applicability to the Pakistani population. This is because the existing solutions fail to completely weigh in the Pakistani correction factors with regards to the environment and life style can only be corrected if there is an measuring tool specific to the Pakistani demographics [11]. Therefore, the objective of our study is to compare EKFC, CKD-EPI 2021, CKD-EPI 2009, CKD-EPI Pak and MDRD equations taking CrCl as gold standard in order to evaluate these equations and understand which equation holds the greatest value when it comes to diagnosing CKD in the Pakistani population.

Materials and Methods

Study Design and Settings

This study was a retrospective cross-sectional study conducted at the Chemical Pathology branch of the Department of Pathology and Laboratory Medicine and the Nephrology unit at The Aga Khan University in Karachi.

The integrated laboratory management system (iLMS) provided consecutive CrCl test results for individuals above the age of 18 for the three months of December 2021 to February 2022.

Study Participants

After excluding individuals below 18 years of age and those above 70 years of age, a total of 2310 results were evaluated in the final dataset. Participants included individuals above the age of 18, and demographic characteristics such as age and gender were noted. The rationale behind the selection of the age criteria was based on the reasoning that the CKD-EPI Pak equation was originally made and validated for this age group [11]. Biochemical results of SCr and CrCl were also recorded. The study sample was obtained from laboratory records, and the data was analyzed retrospectively. The eGFR values for each patient was calculated using the five different equations as described in Table 1.

Laboratory Analysis

The rate-Jaffe reaction was employed for SCr analysis using the Siemens ADVIA 1800 analyzer, which can be linked to a reference method for isotope dilution mass spectrometry (IDMS). The laboratory was accredited by the College of American Pathologists (CAP), and analysis was performed following CLSI guidelines. Normal reference intervals for SCr were determined as 0.9–1.3 mg/dL for males and 0.6–1.1 mg/dL for females.

Data Analysis

Version 22 of the Statistical Package of Social Sciences (SPSS) was used for data analysis. Deming regression analysis was conducted in contrast with CrCl. A threshold of CrCl < 60 mL/minute/1.73 m2 was employed to evaluate the effects of the formulas. The Kruskal-Wallis test was used to compare the medians of Cr, CrCl, MDRD, CKD-EPI 2009, CKD EPI 2021, CKD-EPI Pak & EKFC across various GFR stages. Sensitivity, specificity, positive predictive values, and negative predictive values were determined for each equation. Mean differences

between CKD EPI 2021, CKD EPI 2009, CrCl & EKFC were examined using the Bland-Altman plot.

Table 1: Equations for estimating glomerular filtration rate in adults.

Type of Equation	Formula and criteria		
CrCl	Urine Cr Conc x urinary volume x 1.73/serum Cr x 1440 x BSA		
MDRD	$175 \times SCr^{-1.154} \times age^{-0.203} \times (0.742$ if female$)$		
CKD-EPI 2009	If $SCr \leq 0.9$ (for male): $141 \times (SCr/0.9)^{-0.411} \times 0.993^{age}$ If $SCr > 0.9$ (for male): $141 \times (SCr/0.9)^{-1.209} \times 0.993^{age}$ If $SCr \leq 0.7$ (for female): $144 \times (SCr/0.7)^{-0.329} \times 0.993^{age}$ If $SCr > 0.7$ (for female): $144 \times (SCr/0.7)^{-1.209} \times 0.993^{age}$		
CKD-EPI Pak	$0.686 \times \text{CKD-EPI}^{1.059}$		
CKD-EPI 2021	$142 \times$ minute $(S_{cr}/\kappa, 1)^{\alpha} \times$ max $(S_{cr}/\kappa, 1)^{-1.200} \times 0.9938^{age} \times 1.012$ [if female] where: S_{cr} = standardized serum creatinine in mg/dLκ = 0.7 (females) or 0.9 (males) α = -0.241 (female) or -0.302 (male) min $(S_{cr}/\kappa, 1)$ is the minimum of Scr/κ or 1.0 max $(S_{cr}/\kappa, 1)$ is the maximum of Scr/κ or 1.0. age (years)		
EKFC	Age (years)	SCr/Q	Equation for eGFR
	2 – 40	<1	$107.3 \times (Scr/Q)^{-0.322}$
		≥1	$107.3 \times (Scr/Q)^{-1.132}$
	>40	<1	$107.3 \times (Scr/Q)^{-0.322} \times 0.990^{(Age-40)}$
		≥1	$107.3 \times (Scr/Q)^{-1.132} \times 0.990^{(Age-40)}$

BSA: Body surface area

For EKFC Equation:

Scr: Serum creatinine concentration

Q value calculations for ages 2–25 years:
Males: $\ln(Q) = 3.200 + 0.259 \times Age - 0.543 \times \ln_{(Age)} - 0.00763 \times Age^2 + 0.0000790 \times Age^3$
Females: $\ln(Q) = 3.080 + 0.177 \times Age - 0.223 \times \ln_{(Age)} - 0.00596 \times Age^2 + 0.0000686 \times Age^3$

Q value calculations for ages >25 years:
Males: Q = 80 µmol/L (0.90 mg/dL)
Females: Q = 62 µmol/L (0.70 mg/dL)

Results

In this retrospective cross-sectional study, a cohort of 2310 participants, comprising 1,075 females (46.5%) and 1,235 males (53.5%), with a median age of 52 years, was analyzed. The study evaluated CrCl and its comparison with six equations (EPI-2009, MDRD, EPI-2021, EPI-Pak, CKD-EPI 2012, and EKFC) for estimating Glomerular Filtration Rate (GFR) across five stages of kidney function (GFR stages I-V).

Table 2: Comparison of CrCl and the 6 formulae in the 5 GFR stages (n = 2,310).

GFR Stages	N	CrCl (mL/ minute)	EPI-2009 (mL/minute)	MDRD (mL/ minute)	EPI-Pak (mL/minute)	EPI-2021 (mL/minute)	EGFR (EKFC) (mL/ minute)	p-value
	2,310	60.00 (34.00-89.60)	71.07 (42.53-100.32)	63.87 (39.34-91.33)	62.77 (36.40-90.32)	72.28 (43.25-102.07)	69.72 (42.72-95.83)	<0.001
I	578	111.00 (100.00-131.00)	109.11 (99.94-122.43)	106.61 (90.09-131.69)	98.78 (90.06-111.66)	110.11 (96.74-121.47)	105.38 (96.07-116.48)	<0.001
II	578	75.00 (67.00-81.00)	87.99 (78.98-100.32)	75.97 (67.18-87.15)	78.70 (70.19-90.32)	86.73 (68.21-102.06)	85.77 (77.15-95.83)	<0.001
III	669	45.00 (37.00-52.00)	54.23 (46.15-62.73)	50.17 (42.07-57.67)	47.14 (39.73-55.00)	56.48 (44.18-73.58)	53.37 (46.34-61.15)	<0.001
IV	281	22.00 (19.00-26.00)	28.52 (24.85-33.33)	26.90 (22.72-32.64)	23.87 (20.60-28.12)	30.55 (23.76-41.77)	29.31 (25.45-34.52)	<0.001
V	204	9.00 (6.00-12.00)	11.17 (7.71-14.95)	11.08 (7.61-14.89)	8.84 (5.97-12.04)	11.16 (7.52-16.84)	12.11 (8.66-16.06)	<0.001

Results presented in Table 2 indicate that EPI-2009 and EPI-2021 exhibit the closest agreement with CrCl across all stages, as evidenced by their smallest mean differences compared to CrCl within each stage. It is noteworthy that as GFR stages decrease, indicating lower kidney function, mean differences between CrCl and all formulas tend to increase, suggesting potential decline in formula accuracy in individuals with reduced kidney function.

Table 3: Diagnostic ability of CrCl versus MDRD, EPI 2009, EPI-Pak, EPI 2021 & EKFC.

	MDRD	EPI 2009	EPI-Pak	EPI 2021	EKFC
Sensitivity	90.99%	95.15%	95.15%	83.10%	94.63%
Specificity	88.58%	94.55%	94.55%	83.22%	95.07%
PPV	88.83%	94.57%	94.57%	83.17%	95.04%
NPV	90.78%	95.13%	95.13%	83.15%	94.66%
Agreement (%)	89.78%	94.85%	94.85%	83.16%	94.85%
R^2 value	0.5834	0.8433	0.8439	0.4564	0.8386

Next, we assessed the diagnostic ability of the six equations and compared it with CrCl for all the individuals. Among the equations assessed, EPI-Pak demonstrates the highest percentage of agreement and sensitivity of 94.85% and 95.15% respectively, while EKFC exhibits the highest specificity and positive predictive value (PPV) of 95.07% and 95.04% respectively, and EPI 2009 shows the highest negative predictive value (NPV) of 95.13%. Remarkably, the EPI-Pak equation emerges with the highest R2 value, indicating superior assessment of kidney function compared to other formulas as depicted in Table 3.

Finally, we also highlighted the agreement between CrCl and individual equations in the form of Bland-Altman plots in Figures 1-5 which also highlights that EPI-Pak is perhaps the most suited when it comes to assessing the renal function in the Pakistani population. In figures 6 and 7, we try to compare the median values of the three important equations i.e., EPI-Pak, EPI2009 and EPI2021 with factors like gender, CKD stage and age-groups which can help us draw important conclusions.

Figure 1: Bland Altman plot comparing CrCl and MDRD equation.

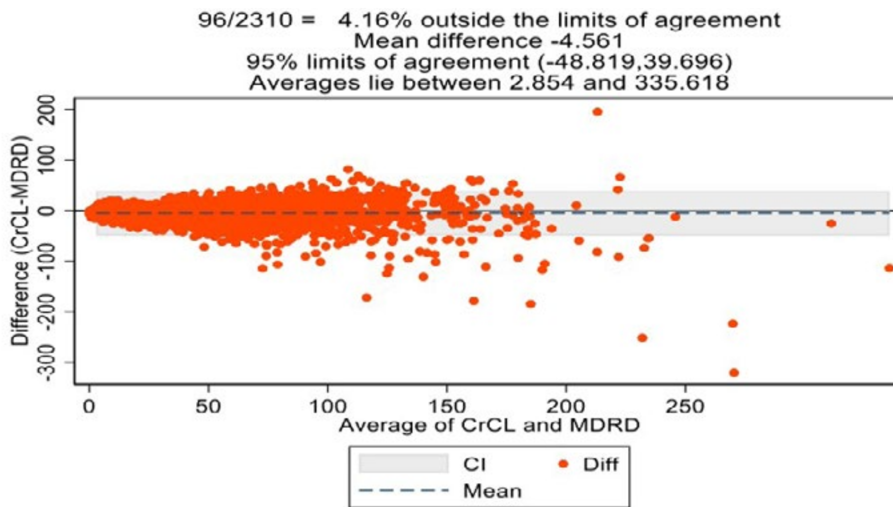


Figure 2: Bland Altman plot comparing CrCl and EPI 2009 equation.

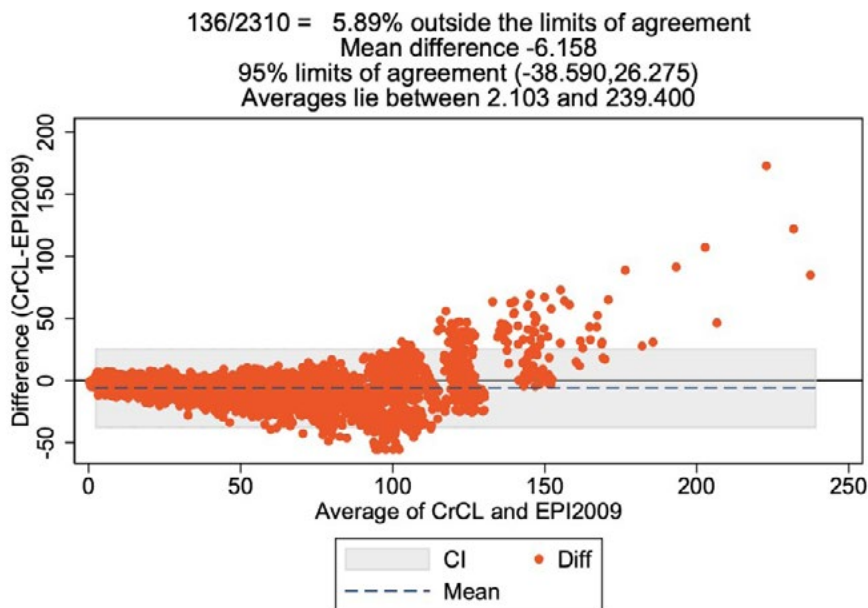


Figure 3: Bland Altman Plot comparing CrCl and EPI-Pak equation.

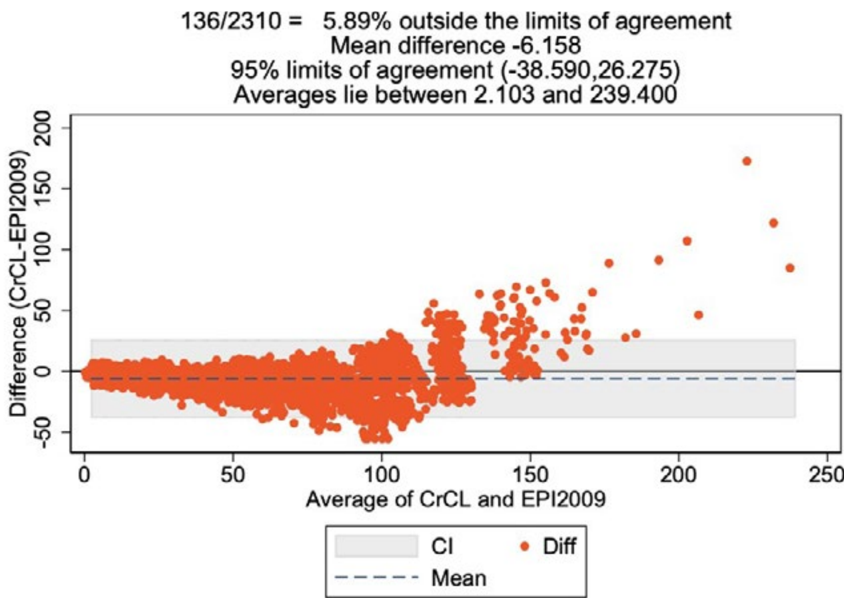


Figure 4: Bland Altman Plot comparing CrCl and EPI2021 equation.

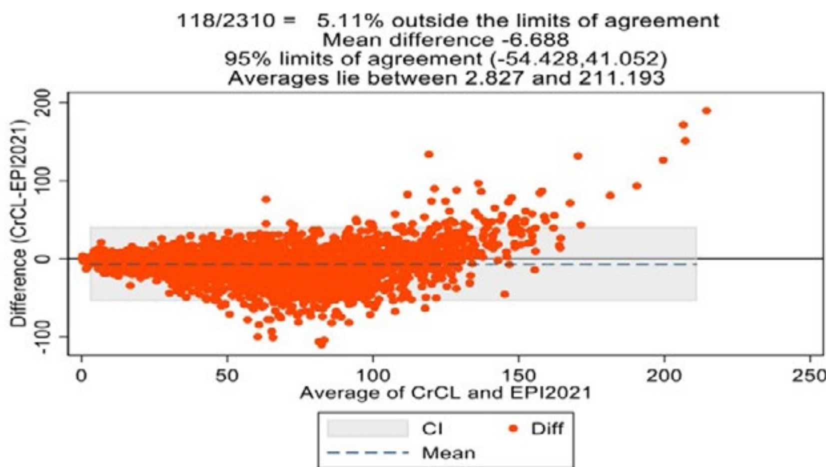


Figure 5: Bland Altman Plot comparing CrCl and EKFC equation.

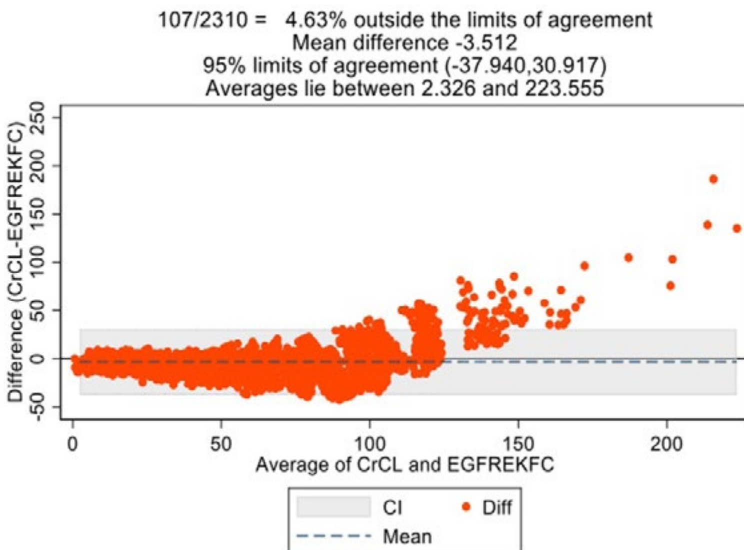


Figure 6: Comparison of median values of EPI-Pak, EPI2009 and EPI2021 equations with gender and CKD stage.

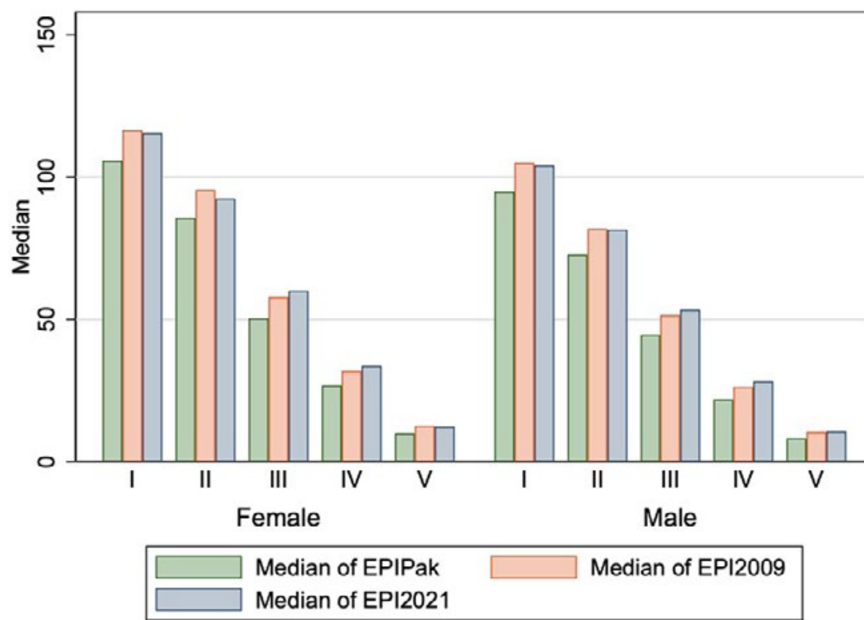
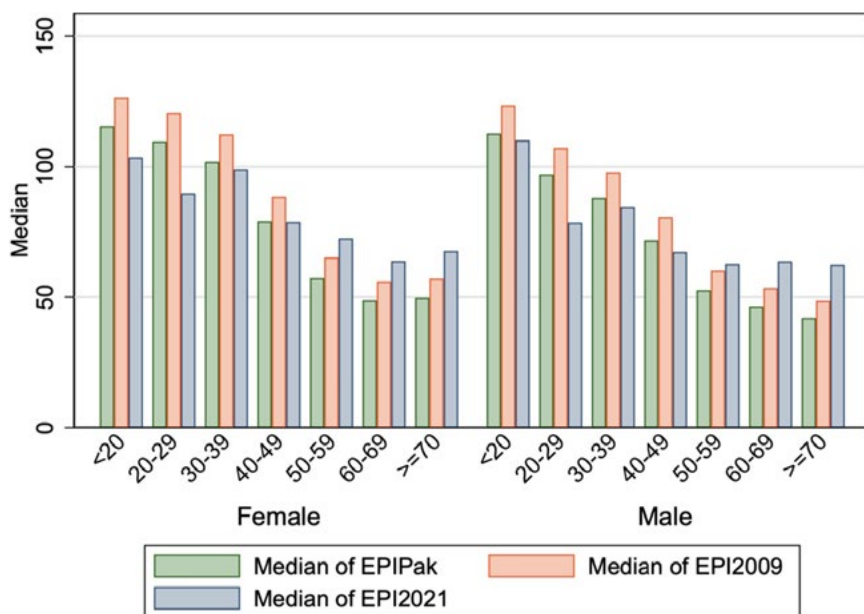


Figure 7: Comparison of median values of EPI-Pak, EPI2009 and EPI2021 equations with gender and age-group.



Discussion

CKD is a growing concern in middle- and low-income countries and more importantly, in Pakistan. Timely analysis, clinical staging and outcomes are used to decide on the mode of therapy for the patient on a case-by-case basis. All these modes of treatment have relevant financial concerns associated with them and therefore timely diagnosis of the disease and its extent has become very important [12]. To achieve these values as accurately as possible, a plethora of equations and formulas have been derived, as mentioned earlier more, and each successive method was aimed at addressing the gaps in their predecessor methods.

In this retrospective cross-sectional study, we used a Pakistani sample to analyze all the mentioned equations to gauge the sensitivity and specificity in diagnosing CKD and its staging when it comes to the demographics of Pakistan. It is quite evident from Table 3 and the Bland Altman plots that EPI-Pak outperforms all the equations and more importantly, the newly presented, EKFC formulae owing to its better reliability and better assessment of kidney function and having the highest R2 value. On the other hand, EKFC excels when it comes to a higher specificity and better positive predictive value while keeping up with EPI-PAK and EPI-2009 when it comes to agreement percent. This shows it is just as reliable for sorting

out people without CKD or End-Stage Renal Disease (ESRD) and for being used as an evaluation standard by using creatinine clearance. Our findings align with those Safdar et al and Ahmed et al, who pointed out that the EPI-Pak equation is perhaps the most accurate and precise equation when it comes to estimating eGFR in the South-Asians and appropriate measures should be taken for its implementation in the clinical laboratories [8, 11]. When talking about relatively older equations such as MDRD, it is evidently clear through many other studies as well that it has been outperformed by all its successors, and compared to MDRD, CKD-EPI gave the best estimation of eGFR [13]. Once in the top tiers, the CKD-EPI equations were put to question by another Pakistani study, namely Ahmed et al which proved that the EPI-Pak equation was perhaps more suitable to assessing CKD in the Pakistani population than the CKD-EPI 2021 equation [14]. Finally, the recently developed equation of EKFC attempted to estimate the eGFR using a creatinine-based equation and literature shows that this equation improved the accuracy of eGFR assessment in cohorts from Europe, the United States, and Africa [15]. Our study, with its results, shows that this might not apply the same way to a Pakistani cohort. EPI-Pak, which had a sensitivity of 93.2% in the study by Ahmed et al. now had a sensitivity of 95.15% in our study, with EKFC's sensitivity at 94.63%. Although the difference might be negligible, these results assert importance of EPI-Pak equation being the best option when it comes to estimating eGFR for a Pakistani cohort. Implementing the EPI-Pak equation in clinical laboratories could enhance the accuracy of CKD diagnosis and improve patient outcomes in Pakistan

Finally, like all other studies, there are some limitations to this study as well. These limitations involve the inherent restrictions when it comes to using eGFR because when compared with the results of measured GFR (mGFR) which shows a concerning 38% subjects being misclassified in their GFR groups based on their CKD stage. Furthermore, factors such as finite data on mGFR boundaries for age, ethnicity and gender along with variations in markers of mGFR also create a gap in arriving at an actual 100% reliable value [16]. The study's retrospective cross-sectional design may introduce several potential biases. For instance, selection bias is a concern, as the sample only includes participants from a single institution, possibly limiting the generalizability of the findings to the broader Pakistani population. Additionally, information bias could arise from the reliance on existing medical records, which may contain inaccuracies or incomplete data. Finally, our study also used the 24-hour urine CrCl as the gold standard which actually tends to overestimate the GFR [17].

As evident by the findings in this study, we can conclude that an automated reporting of eGFR using CKD-EPI Pak equation in laboratories across Pakistan will prove beneficial for the physicians as well as the patients for an accurate and timely diagnosis. These findings can also be shared with clinical laboratories in Pakistan and neighboring countries to facilitate reporting of eGFR when serum creatinine is measured which will pave the way for better clinical outcomes. In order to overcome the possible bias associated with retrospective designs, future studies should consider a prospective design and include a larger, more diverse sample to validate these findings.

Declaration of Conflict of interests

The authors of this article declare that there is no conflict of interest with regard to the content of this manuscript.

Ethical Considerations

The study was conducted in accordance with ethical guidelines and was approved by the institutional ethical review committee (Approval No: 2022-7451-21323). Anonymity of participants was preserved by coding the study identifiers into medical record numbers.

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Case Report

Urinary findings in a 12-year-old child, a rare case of Follicular Cystitis

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Keywords

Pediatrics; Follicular Cystitis; Chronic Cystitis; Urinary Sediment; Urothelial cells

Abstract

Follicular cystitis (FC) is a chronic form of cystitis with uncertain etiology, characterized by the presence of lymphoid follicles in the bladder mucosa as a result of chronic irritation. This can be caused by various factors such as prolonged catheterization, lithiasis, recurrent urinary tract infections or neoplastic bladder pathology. Although it is a rare pathology, it is mainly seen in women over 50 years of age and manifests with nonspecific urinary symptoms such as dysuria, pollakiuria, haematuria and suprapubic pain. We describe a case of a 12-year-old boy with dysuria, haematuria and hypogastric pain. Despite the absence of a history of lithiasis or trauma, and no bacteria found in urinalysis, erythrocytes and leukocytes were found, along with reactivated and degenerated urothelial cells accompanied by heterogeneous-sized cells with a high nucleus/cytoplasm ratio. Ultrasonography showed no abnormalities, but cystoscopy revealed irregularities in the trigone of the bladder and biopsy confirmed the presence of lymphoid follicles, characteristic of FC. This case underscores the relevance of considering FC in patients with persistent bladder irritation and recurrent haematuria. Cystoscopy and histologic evaluation are crucial for an accurate diagnosis, although the role of the clinical laboratory is limited, an experienced specialist can facilitate a proper diagnosis.

Introduction

Follicular cystitis (FC) or cystitis follicularis is a type of chronic cystitis of uncertain etiology, characterized by the presence in the submucosal connective tissue of large numbers of plasma cells and lymphocytes that organize themselves to form lymphoid follicles with germination centers inside. This pathology develops from a chronic irritation of the bladder mucosa that will later give rise to histopathological lesions that characterize it [1, 2].

Chronic irritation of the bladder mucosa may be due to various factors such as prolonged bladder catheterization, lithiasis, repeated urinary tract infections (UTI) or neoplastic bladder pathology. In response to these stimuli, the bladder mucosa responds by the formation of lymphoid follicles characteristic of this pathology. In addition, it has been suggested that the bladder mucosa of patients with FC may have the capacity to

secrete immunoglobulins [1, 2].
The frequency of this pathology is very low, which is evidenced by the few cases that have been published. In these reports, there is a predominance of the female sex and a higher occurrence in those over 50 years of age [1-3].
FC leads to nonspecific symptoms in the urinary tract. It usually presents with dysuria, polyuria, haematuria (microscopic or macroscopic) or suprapubic pain in the lower urinary system [1-3].

Case report

A 12-year-old boy with dysuria, haematuria and pain in the hypogastrium. There was no history of trauma and no family history of urinary lithiasis. On physical examination the patient's vital signs were stable. The abdomen was not distended. In the anamnesis, the patient refers to having few urinary voidings per day and heat stroke every summer.
On suspicion of a UTI, a urine study and urine culture was requested. The dipstick detected the presence of protein in the urine, which was quantified by turbidimetry, erythrocytes and leukocytes (Table 1). The urinary sediment showed microhaematuria (28 erythrocytes/µL), leukocyturia (222 leukocytes/µL), absence of bacteriuria which was later confirmed by urinary culture and, in addition, the presence of urothelial cells with reactive and degenerative changes (Figure 1) accompanied by heterogeneous-sized cells with a high nucleus/cytoplasm ratio (Figure 2), whose morphology was verified with another sample the following day.

Table 1: Laboratory test results.

Laboratory test	Results
Dipstick	
pH	5.0
Glucose	0 (Normal)
Proteins	100 mg/dL
Erythrocytes (hemoglobin/myoglobin)	20/µL
Ketone Bodies	Negative
Bilirubin	Negative
Urobilinogen	Negative
Nitrites	Negative
Leukocytes (leukocyte esterases)	25/µL
Density	1.023
Urinary sediment	
Erythrocytes	28 erythrocytes/µL
Leukocytes	222 leukocytes/µL
Bacteria	Absence
Urothelial cells	Abundant
Urine culture	Negative
Protein/creatinine (Cr) ratio	956.1 mg/g Cr

Figure 1: Urothelial cells with reactive and degenerative changes (phase contrast, x400).

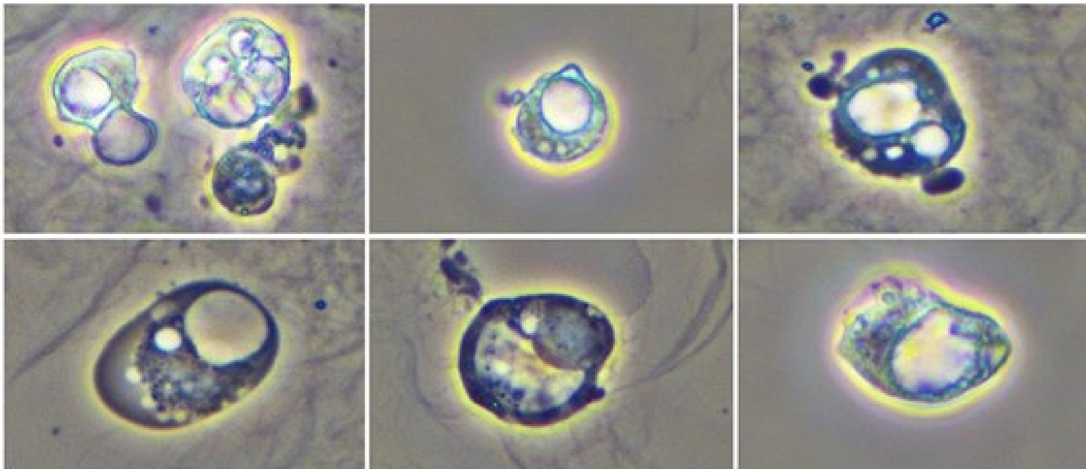
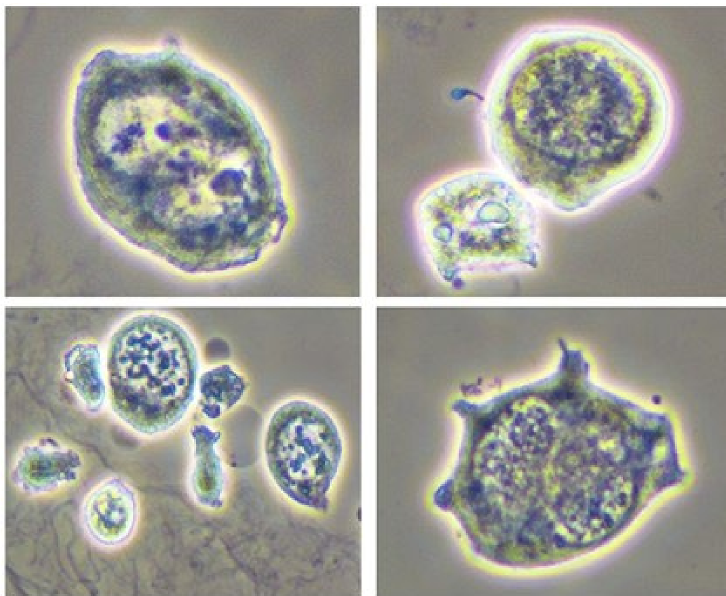


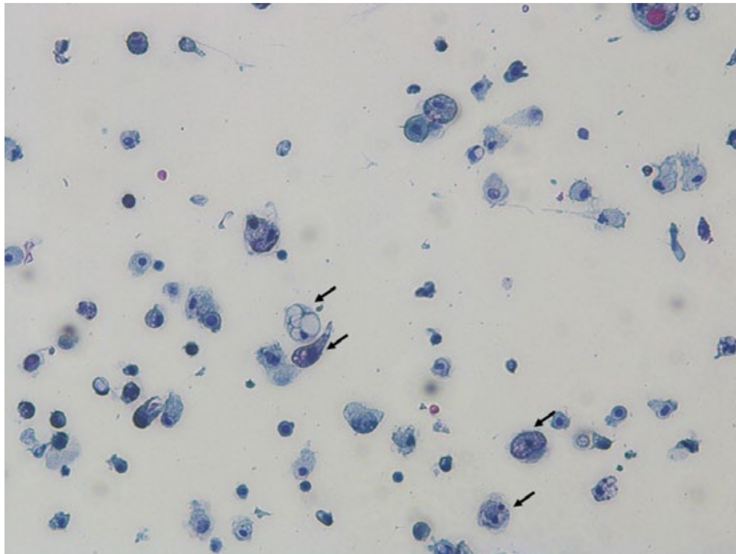
Figure 2: Heterogeneous-sized cells with a high nucleus/cytoplasm ratio (phase contrast, x400).



In view of the laboratory findings, an ultrasound of the urinary system was performed, in which no abnormality was found, and urine cytology was requested.

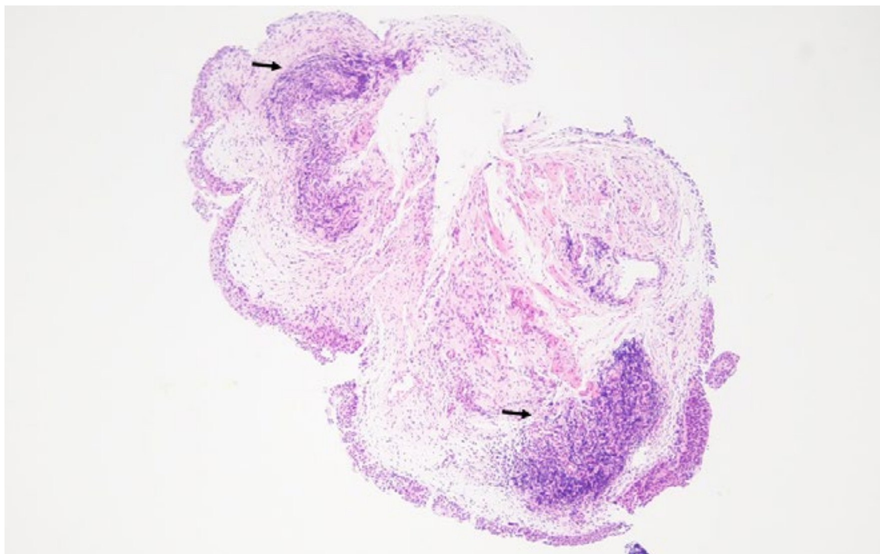
The cytology performed by the Pathology Department confirmed the presence of degenerated urothelial cells (Figure 3) and, in addition, the presence of some cells with anisokaryosis, nuclear atypia and nucleolus, for which a cystoscopy was recommended.

Figure 3: Cytology showing urothelial cells with reactive and degenerative changes (arrow) and other inflammatory cells in the background (Papanicolau stain, x200).



Cystoscopy revealed superficial irregularities in the trigone area and biopsies were taken from this area. The existence of lymphoid follicles with germinal center formation, which is pathognomonic for FC, was finally verified by the biopsy results (Figure 4).

Figure 4: Bladder biopsy showing two lymphoid follicles (arrow) with germinal centers in subepithelial area consisting of the majority of lymphocytes and plasma cells (Hematoxylin-eosin, x40).



Due to the lack of common risk factors, such as prolonged bladder catheterization, lithiasis, repeated UTI or neoplastic bladder pathology, this case report represents a rare case of FC. The patient's poor hydration and urinary habits may have contributed to the chronic bladder irritation that ultimately resulted in the development of FC.

In the treatment plan, the control of inflammation and cause-oriented therapy are the main goals. As a result, it was advised that the patient drink more water and void more frequently each day. The patient had a clinically significant improvement at the follow-up appointment showing a good health status and no recurrence of haematuria.

Discussion

FC usually presents with haematuria (microscopic or macroscopic) and dysuria [1-3]. These symptoms and findings, however, are nonspecific and just confirm the clinician's suspicions. While a patient's physical examination may

sometimes be normal, nonspecific bladder discomfort is rarely seen.

Laboratory findings have limited value in the diagnosis of FC, unless the urine analysis shows characteristic urothelial cells with reactive and degenerative changes as well as increased number of lymphocytes varied in maturation with a predominance of small mature lymphocytes accompanied by immature (follicular center) lymphocytes including tingible body macrophages and follicular dendritic cells [3, 4]. Furthermore, this characteristic pleomorphism of the lymphoid population sets it apart from both the majority of cases of non-Hodgkins lymphomatous UT involvement, where the dispersed atypical lymphoid cells are relatively uniform, and chronic cystitis with increased lymphocytosis, where there is a dispersion of mature lymphocytes and other chronic inflammatory cells in the background [5].

Imaging methods provide a little role in the diagnosis of FC. However, ultrasonography may be useful in some cases of follicular cystitis with the appearance of a papillary-type pseudoneoplastic mass [6]. Thus, imaging methods may aid in the differential diagnosis rather than in the diagnosis of the patient.

The differential diagnosis of cytologic material should consider other pathologies. These include granulomatous cystitis, interstitial cystitis, lymphoma, high-grade urothelial carcinoma and lymphoepithelial carcinoma. These pathologies can present cytologic patterns that vary significantly.

In granulomatous cystitis, loose clusters of epithelioid histiocytes with elongated nuclei are observed [7] and in interstitial cystitis, the presence of inflammatory cells, mainly neutrophils and occasionally eosinophils, is common [8].

In lymphoma, the cell population is discohesive and monomorphic, composed of large, atypical lymphocytes with a high nucleus/cytoplasm ratio, irregular nuclear borders and prominent nucleoli [9].

Finally, in lymphoepithelial carcinoma and high-grade urothelial carcinoma, a heterogeneous population of lymphoid cells is observed in the background along with carcinomatous cells exhibiting a high nucleus/cytoplasm ratio, irregular nuclear membranes, and coarse chromatin [10].

Cystoscopic findings can be detected, mainly the presence of nodules with erythematous surface and trigonal location. Cystoscopy followed by histological examination is currently the gold standard for diagnosis of FC [2-4]. The presence of lymphoid follicles in the bladder wall with germinal center formation is pathognomonic for FC.

There is no identified specific treatment; generally treatment is targeted at the cause and suppression of the inflammation. In addition, anti-inflammatory drugs are also routinely used to reduce the inflammatory reaction. Prednisone treatment and vitamin A supplementation are other conservative treatment options for the reduction of inflammation.

Lessons learnt

- This article highlights the importance of urinalysis performed by a skilled clinical laboratory specialist. Urinalysis is normally used to detect different pathologies, such as UTI, crystalluria, monitor chronic kidney disease, evaluate tubular disorders, but it can also contribute to the diagnosis of less frequent pathologies, such as FC.
- FC is a rare condition mainly described in adult women, but it should be considered in any patient, regardless of age of sex, who presents with persistent bladder irritation, recurrent haematuria and urothelial cells with reactive and degenerative changes in the urinary sediment, especially after ruling out more common pathologies.
- The guideline for diagnosing FC involves a comprehensive approach starting with a thorough patient history and physical examination to identify symptoms such as dysuria, hematuria, and bladder pain, alongside risk factors like poor hydration and urinary habits. Key laboratory tests include urinalysis to detect proteinuria, hematuria, and leukocyturia, and urine culture to rule out bacterial infections. Urine cytology is crucial for identifying urothelial cells with reactive and degenerative changes. Imaging studies, such as ultrasound, are used to exclude other abnormalities. If FC is suspected, cystoscopy is performed to inspect for bladder irregularities, particularly in the trigone area, followed by biopsies of suspicious areas. The definitive diagnosis is confirmed through histological examination of biopsy samples, identifying lymphoid follicles with germinal centers, which are pathognomonic for FC.
- There is no specific treatment identified for follicular cystitis; generally, treatment focuses on addressing the underlying cause and suppressing inflammation. Additionally, anti-inflammatory drugs are routinely used to mitigate the inflammatory response.

Author Contributions

All authors confirmed they have contributed to the intelectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors' Disclosures or Potential Conflicts of Interest

No authors declared any potential conflicts of interest.

Consent

Informed and written consent was obtained from parent.

Ethical approval

Not applicable.

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Brief report

Continuous reference intervals for plasma cystatin C and creatinine in Vietnamese children

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Keywords

continuous reference intervals, plasma, cystatin C, creatinine, children, renal function.

Abstract

Background

Serum (plasma) creatinine and cystatin C are widely used in pediatric clinical practice to assess glomerular filtration rate. Both markers have limitations due to the low index of individuality, which affects the clinical sensitivity of population-based reference intervals, especially when wide age ranges are considered. This study aimed to establish age-related reference intervals for plasma cystatin C and creatinine in Vietnamese children.

Methods

A total of 454 children, equally divided between boys and girls, aged from 1 day to 18 years, were recruited from the outpatient clinic of Vietnam National Children's Hospital. None of the participants had kidney or infectious diseases. Plasma samples were analyzed for cystatin C and creatinine using standard clinical chemistry methods. Using the the Lambda-Mu-Sigma method, we derived centile charts showing dynamic changes in these biomarkers.

Results

In this cohort, plasma creatinine levels were high at birth, declined to their lowest point between ages of 2 and 3 years, and then gradually increased until adulthood. Plasma cystatin C levels were also elevated at birth, decreased to a steady state around age of 2 year, and remained stable until age of 10 years. From ages 10 to 14 years, cystatin C levels slightly increased, followed by a decrease from ages 15 to 18 years.

Conclusions

Accurate assessment of glomerular filtration in children requires reliable laboratory tests and age-specific reference intervals. Providing serum (plasma) cystatin C and creatinine reference intervals with appropriate age partitions is crucial for improving the clinical sensitivity for detecting renal dysfunction, especially during the first few years of life.

Introduction

Evaluation of renal function (glomerular filtration) in children plays an important role in many clinical settings. Serum creatinine and creatinine clearance are widely used in pediatric clinical practice to assess glomerular filtration rate. However, serum creatinine concentrations are affected by muscle mass, age, and diet, which can confound its assessment of glomerular filtration in children [1, 2]. An alternate surrogate biomarker for glomerular filtration is cystatin C. Cystatin C is a low molecular weight serine protease inhibitor that is produced by all human nucleated cells at a steady rate. Cystatin C is freely filtered through the glomerular membrane and is mostly reabsorbed and catabolized by the proximal tubule cells of the kidney. Unlike creatinine, it is not affected by factors such as diet or muscle mass [3, 4]. An equation for estimated glomerular filtration rate in children has been described for cystatin C, which facilitates improved glomerular filtration assessment for this population [5].

A study comparing the biological variation of serum cystatin C and serum creatinine in children showed that the within-subject variations of these two markers are similar and suggested that they are both suitable for monitoring renal function in children [6]. However, the within-subject biological variation is small relative to the between-subject biological variation (i.e., low index of individuality) for both serum creatinine and cystatin C. When population-based reference intervals are applied to individual patients, they are much wider than the within-subject biological variation of the patient, and a relatively large pathology or abnormality may be required for the patient result to exceed the reference intervals [6]. In other words, the serum cystatin C or serum creatinine of an unwell child may have to deviate significantly from his physiologic set point before exceeding the population-based reference intervals. This limitation is particularly pronounced when a reference interval with a wide age interval is adopted since the reference limits may be widened to accommodate larger age-related changes.

To overcome this limitation, continuous age-related reference intervals that closely describe the underlying dynamic distribution may be adopted to improve their clinical sensitivity [7-9]. In this study, we measured plasma cystatin C and creatinine, and described the continuous, age-related reference intervals in a cohort of Vietnamese children.

Subjects and methods

A total of 454 children with equal numbers of boys and girls, aged from 1 day to 18 years, who attended the outpatient clinic of Vietnam National Children's Hospital between December 2020 and June 2021, and did not present with kidney disease or infectious disease, were recruited in this study. The exclusion criteria were as follows: 1) patients with nephropathy, digestive system diseases, acute or chronic infections, metabolic or nutritional diseases, autoimmune diseases, thyroid diseases, blood disorders, heart diseases, malignant tumors, burns, muscle damage, obesity or weight loss, or hypertension; 2) patients with blood transfusion; 3) preterm neonates. The children's parents provided informed consents following explanation of the study protocol, which had been approved by the local ethics committee (2374/BVNTW-HĐĐĐ) and complied with the Helsinki Declaration. Venous blood samples were collected into heparin tubes. Samples were centrifuged at 5000 rpm for 5 min, plasma was separated into 1.5 ml tube (Eppendorf). Residual plasma (stored at -80oC) from these children was subjected to cystatin C and creatinine measurements following routine clinical chemistry testing at the biochemistry laboratory at National Children's Hospital, which is accredited to ISO 15189 by the Bureau of Accreditation in Vietnam.

Plasma cystatin C was measured using the Tina-quant Cystatin C Gen.2 on the Cobas c501 platform (Roche Diagnostics, Hanoi, Vietnam), according to manufacturer instructions. This measurement procedure was traceable to the National Institute of Standards and Technology Standard Reference Material 909b Level 2. The analytical measurement range of this measurement procedure was 0.40–6.80 mg/L. The coefficient of variations for within-run and between-run imprecision were <2.2% based on three quality control samples with cystatin C concentrations between 1.11 and 4.14 mg/L. Plasma creatinine was analyzed using a kinetic uncompensated Jaffe method on the AU5800 platform (Beckman Coulter Inc., Hanoi, Vietnam). This creatinine measurement procedure was traceable to the Isotope Dilution Mass Spectroscopy method via National Institute of Standards and Technology Standard Reference Material 967. The analytical measurement range of the serum creatinine measurement procedure was 18- 2200 μmol/L. The coefficient of variation for within-run and between-run imprecisions of this measurement procedure derived from quality control samples was <2%.

The plasma cystatin C and creatinine measurements against age were visualized on scatter plots, which did not reveal any gross outlier. There was no statistically significant difference (two tailed student t-test, p>0.05) between boys and girls for both measurands and the datasets of both genders were combined. Subsequently, the cystatin C and creatinine datasets were subjected to the Lambda-Mu-Sigma (LMS) method to derive centile charts using the LMS Chartmaker Pro software [10]. For plasma creatinine, subjects below 30 day-olds (n = 21) were excluded from analysis as a good fit could not be achieved in the software due to high variance and relatively low data density. The default Lambda, Mu, Sigma (i.e. L, M, S) parameters were set at 3.0, 5.0, and 3.0 equivalent degrees of freedom, respectively, and represented optimal fitting parameters with lowest deviations following adjustments. Smoothed curves were generated for the 2.5th, 5th, 25th, 50th, 75th, 95th, and 97.5th centiles, respectively.

Results and Discussion

The scatter plots and continuous centile (reference intervals) charts for plasma creatinine and cystatin C of the children are shown in Figure 1. Plasma cystatin C concentrations were high

at birth and declined, reaching a steady state around 2 years of age (Figure 2). The median cystatin C concentrations remained stable throughout up to the age of 10 years, and slightly increased from 10 -14 years of age, then decreased in children aged 15-18 years. On the other hand, plasma creatinine concentrations were elevated after birth and dropped quickly after the newborn period. It subsequently rose continuously until 18 years old (Figure 3). The numerical centile values for plasma cystatin C and creatinine at discrete ages are provided as a Supplemental Tables.

Figure 1: Scatter plot of plasma cystatin C (right y-axis) and creatinine (left y-axis) in Vietnamese children aged 0-18 years.

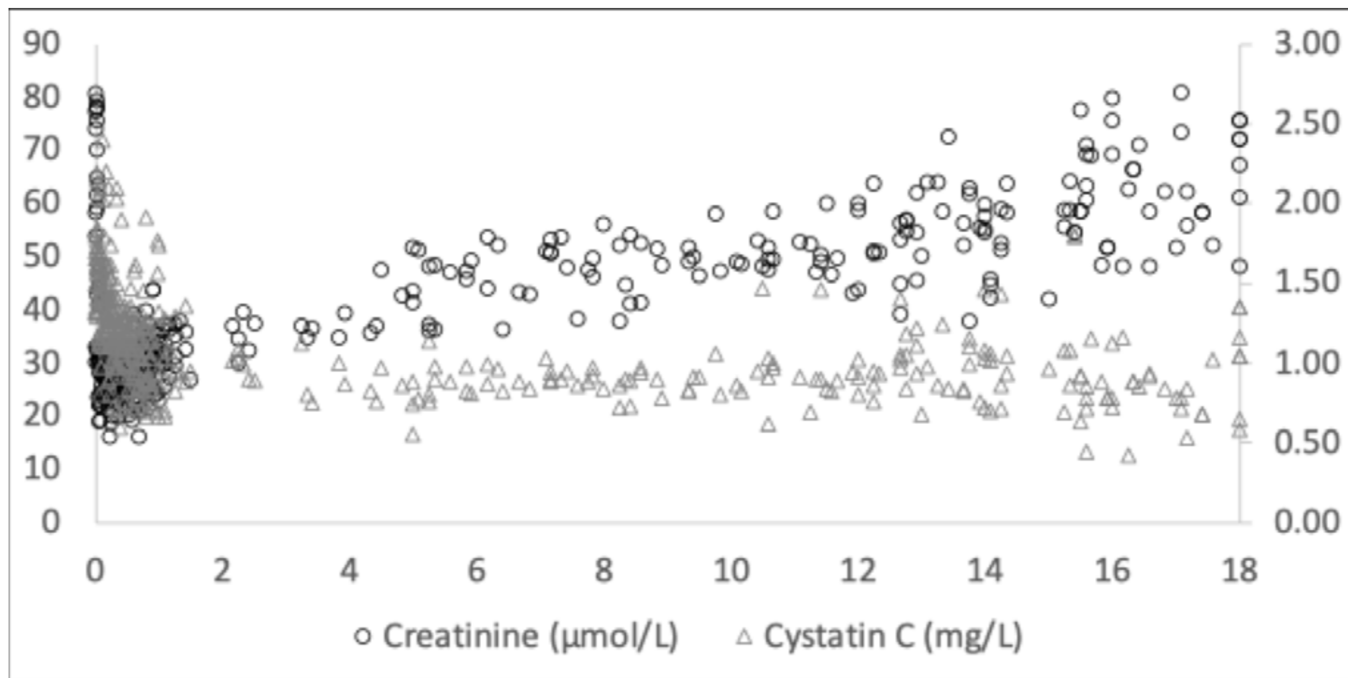


Figure 2: Continuous centile charts presenting 2.5th, 5th, 25th, 50th, 75th, 95th, and 97.5th centiles of plasma cystatin C in Vietnamese children aged 0-18 years.

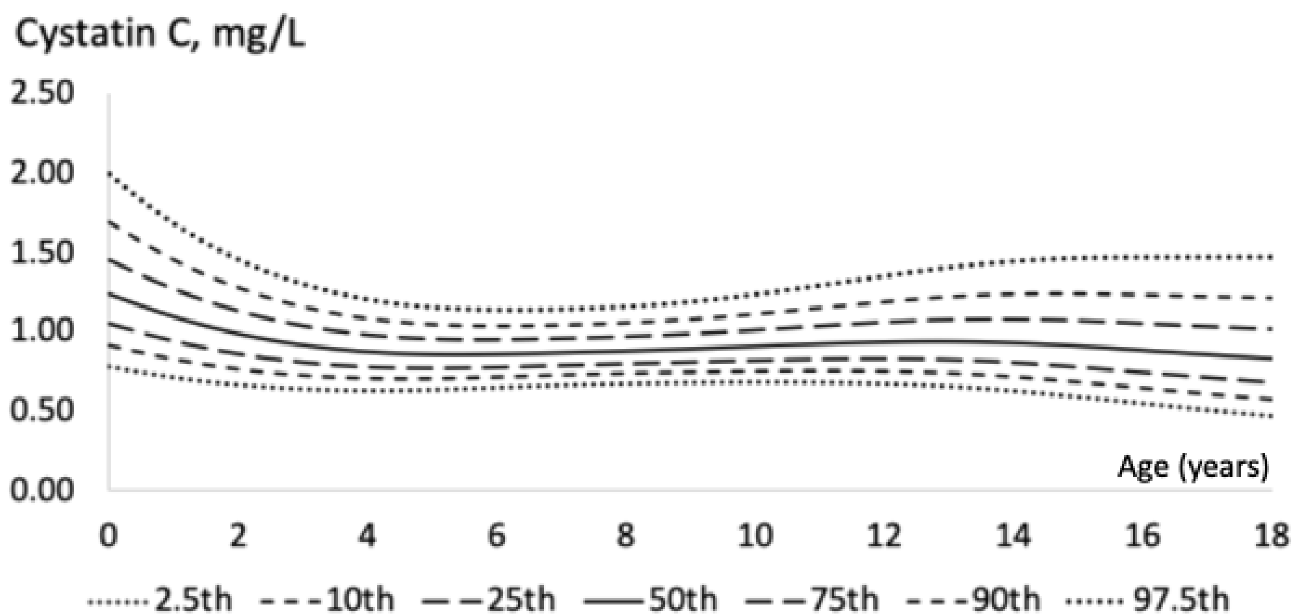
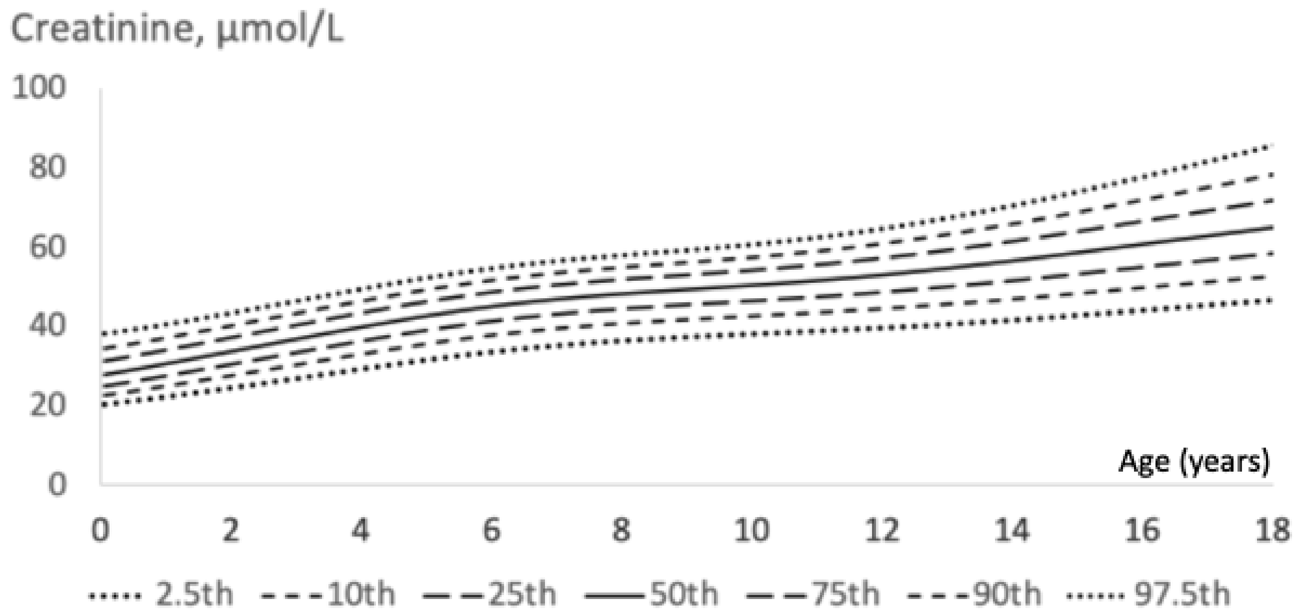


Figure 3: Continuous centile charts presenting 2.5th, 5th, 25th, 50th, 75th, 95th, and 97.5th centiles of plasma creatinine in Vietnamese children aged 0-18 years.



This study provides the reference values for plasma cystatin C and creatinine in a cohort of Vietnamese children without known renal conditions and fills an important knowledge gap for this ethnicity. The plasma cystatin C in this cohort of Vietnamese children was elevated at birth and declined continuously until it stabilizes at 2 years of age, when it remained relatively stable (albeit with a mild increase) until after age 14 when it starts to mildly decline. These findings were largely in line with previous literature although some differences are noted. In this study, we did not find a statistical difference in plasma cystatin C distribution between genders. In contrast, Ziegelasch et al. found gender differences in cystatin C, especially during infancy and puberty in a cohort of 2803 healthy German children. They also reported a mild increase of median cystatin C for both boys and girls at ages 11 to 14 years [11].

At the same time, Liu et al. reported significantly higher serum cystatin C levels in boys aged 4-18 years old compared to girls in a cohort of 4765 healthy Chinese children [12]. Conversely, Cai at al. observed a slight increase in serum cystatin C for males whilst continuous decrease for females after the age of six years in a different cohort of healthy Chinese children [13]. More recently, van Donge et al. found that gender-dependent changes in cystatin C that decreased at birth with age until approximately 2 years, thereafter, increased with age [14]. The differences in the observed dynamic changes in cystatin C in the published studies surveyed above may be related to differences in ethnicity, study population, study design, statistical technique, and sample size.

The plasma creatinine in our Vietnamese cohort was elevated at birth and decline to reach a nadir between ages 2 and 3 years before gradually rising until adulthood. This dynamic change is broadly similar to reports in other populations including Australia, Canada and Germany [7, 9, 15]. Like serum cystatin C, the dynamic change in plasma creatinine concentration throughout childhood suggested a need for age-specific reference intervals for optimal result interpretation in children. Ideally, the age-specific reference intervals should be provided in a continuous manner instead of partitioned by wide age intervals. This consideration is underscored by a study comparing continuous reference intervals for serum creatinine to reference intervals with discrete age partition from the CALIPER study [9]. This study noted a deficiency in representing the age dependence of creatinine concentration with distinct age intervals, which is especially apparent when a child advances across age intervals, e.g., from neonate (0–14 days; 28.3 – 81.4 µmol/L) to infancy (15 days to 2 years; 8.8 – 31.8 µmol/L), where a 3-fold difference in the reference limits may be observed [9].

The key limitations of this study are the relatively small number of children recruited, which may obscure gender-related trends as well as the lack of formal assessment for underlying renal conditions and active infections in the children recruited. These limitations were related to the resource availability of the study team and were mitigated by representative sampling of subjects across the ages with over-representation in the first few years of life where changes are more dynamic and careful case selection from the outpatient clinic.

Conclusion

Proper assessment of glomerular filtration ('renal function') in children is dependent on both the availability of reliable laboratory tests and reference intervals to guide result interpretation. While it is ideal to report continuous reference intervals for measurands that change dynamically with age, it remains a challenge with

most of the laboratory informatics system in use currently. Nonetheless, it is important that serum (plasma) cystatin C and creatinine reference intervals are provided with appropriate age partition to account for the dynamic changes, particularly in the first few years of life to improve the clinical sensitivity for renal dysfunction.

Acknowledgments

We are indebted to the patients and their families for their consent to participate in the study.

Author Contributions

All authors contributed to the development and analysis of this study. Dr. Mai developed the project plan. Dr Ha and Ms. Dung selected participants and perform sample analysis in the Clinical Biochemistry Laboratory, National Children's Hospital, Vietnam. Dr Tze Ping Loh performed the statistical analysis. Dr Mai wrote the first draft of this manuscript and all authors reviewed, edited, and approved the final manuscript.

Conflicts of Interest

None to declare.

Research Funding

None declared.

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Supplemental Data Table 1: Numerical centile values for plasma cystatin C at discrete ages.

Age (year)	Plasma cystatin C concentration (mg/mL)						
	2.5th	10th	25th	50th	75th	90th	97.5th
0	0.7755909	0.9101604	1.051529	1.236179	1.45538	1.687861	1.994763
0.1	0.768464	0.9010174	1.039981	1.221079	1.435502	1.662303	1.960829
0.2	0.7613209	0.8918592	1.028429	1.206006	1.415716	1.636943	1.927287
0.3	0.754196	0.8827255	1.016919	1.191013	1.396086	1.611853	1.894226
0.4	0.7471327	0.8736659	1.005506	1.176168	1.37669	1.587125	1.86175
0.5	0.740173	0.8647284	0.994246	1.161534	1.357602	1.562842	1.829957
0.6	0.7333557	0.8559581	0.9831902	1.14717	1.338892	1.539084	1.798933
0.7	0.7267152	0.8473944	0.9723833	1.133129	1.320617	1.515914	1.768749
0.8	0.7202781	0.8390678	0.9618594	1.119447	1.30282	1.493378	1.739451
0.9	0.7140631	0.8309996	0.9516426	1.106153	1.285531	1.471505	1.711066
1	0.7080805	0.8232016	0.9417458	1.093261	1.268763	1.450307	1.6836
1.1	0.7023333	0.815677	0.9321723	1.080772	1.252517	1.429782	1.657046
1.2	0.696822	0.8084267	0.9229231	1.068688	1.236792	1.409927	1.631391
1.3	0.6915442	0.8014488	0.9139962	1.057005	1.221583	1.390731	1.60662
1.4	0.6864967	0.7947399	0.905388	1.045719	1.206883	1.372185	1.582713
1.5	0.6816757	0.7882959	0.8970938	1.034825	1.192684	1.354275	1.559649
1.6	0.6770771	0.7821128	0.889109	1.024316	1.178977	1.336988	1.537409
1.7	0.6726971	0.7761862	0.8814287	1.014185	1.165753	1.320313	1.515973
1.8	0.6685318	0.7705119	0.8740479	1.004427	1.153002	1.304235	1.49532
1.9	0.6645771	0.7650855	0.8669616	0.9950349	1.140718	1.288743	1.475432
2	0.6608291	0.7599028	0.860165	0.9860027	1.128889	1.273823	1.45629
2.1	0.6572839	0.7549596	0.853653	0.9773238	1.117508	1.259464	1.437875
2.2	0.6539376	0.7502513	0.8474206	0.968992	1.106566	1.245654	1.42017
2.3	0.6507862	0.7457737	0.841463	0.9610009	1.096054	1.23238	1.403156
2.4	0.6478267	0.7415237	0.8357761	0.9533455	1.085966	1.219632	1.386818
2.5	0.6450567	0.7374985	0.8303569	0.9460214	1.076294	1.207402	1.371142
2.6	0.6424736	0.7336952	0.8252019	0.939024	1.067032	1.195678	1.356112
2.7	0.6400748	0.7301108	0.8203074	0.9323483	1.058172	1.184451	1.341715
2.8	0.6378572	0.7267419	0.8156694	0.925989	1.049708	1.173711	1.327934
2.9	0.6358179	0.7235852	0.8112838	0.9199407	1.041633	1.163448	1.314755
3	0.633954	0.7206373	0.8071466	0.9141983	1.033938	1.153651	1.302165
3.1	0.6322625	0.7178949	0.8032538	0.9087565	1.026616	1.144311	1.290148
3.2	0.6307403	0.7153545	0.7996013	0.90361	1.019661	1.135419	1.278693
3.3	0.6293845	0.7130128	0.796185	0.8987536	1.013066	1.126965	1.267785
3.4	0.6281929	0.710867	0.793002	0.8941833	1.006824	1.118941	1.257414
3.5	0.6271634	0.7089151	0.7900493	0.8898953	1.000931	1.111341	1.247568
3.6	0.6262926	0.707153	0.7873226	0.8858841	0.9953799	1.104154	1.238237
3.7	0.6255767	0.7055766	0.7848167	0.8821434	0.990162	1.097371	1.229406
3.8	0.625012	0.7041815	0.7825266	0.8786672	0.9852698	1.090982	1.221063
3.9	0.6245944	0.7029632	0.7804474	0.8754492	0.9806958	1.084978	1.213194
4	0.6243209	0.7019182	0.7785747	0.8724844	0.9764335	1.07935	1.205791
4.1	0.6241887	0.7010434	0.7769051	0.8697683	0.9724771	1.07409	1.198842
4.2	0.6241949	0.7003356	0.7754349	0.8672964	0.9688208	1.069193	1.192338

4.3	0.6243367	0.6997916	0.7741601	0.8650641	0.9654588	1.06465	1.186269
4.4	0.624611	0.6994081	0.7730773	0.8630669	0.9623855	1.060454	1.180627
4.5	0.6250146	0.6991812	0.7721819	0.8612995	0.9595945	1.056598	1.175402
4.6	0.6255431	0.6991062	0.7714685	0.8597555	0.9570782	1.053072	1.170581
4.7	0.6261925	0.6991785	0.7709321	0.858429	0.9548293	1.049868	1.166156
4.8	0.6269592	0.6993942	0.7705681	0.8573147	0.9528415	1.046978	1.162116
4.9	0.6278396	0.6997492	0.7703718	0.8564072	0.9511083	1.044395	1.158452
5	0.6288297	0.7002391	0.7703385	0.8557008	0.9496233	1.042111	1.155155
5.1	0.6299246	0.7008584	0.7704622	0.8551887	0.9483784	1.040117	1.152214
5.2	0.6311138	0.701596	0.7707305	0.8548575	0.9473585	1.038395	1.149609
5.3	0.6323847	0.7024382	0.771129	0.8546911	0.946546	1.036926	1.147319
5.4	0.6337251	0.703372	0.7716436	0.8546742	0.9459238	1.035692	1.145322
5.5	0.6351253	0.7043869	0.7722631	0.8547947	0.9454787	1.034678	1.143602
5.6	0.6365758	0.7054731	0.7729771	0.8550413	0.9451985	1.033871	1.142145
5.7	0.6380678	0.7066212	0.7737756	0.8554033	0.9450715	1.033258	1.140936
5.8	0.6395922	0.7078215	0.7746487	0.8558699	0.9450859	1.032826	1.139961
5.9	0.6411402	0.709065	0.7755865	0.8564308	0.9452308	1.032564	1.139208
6	0.6427038	0.7103431	0.7765801	0.8570764	0.9454957	1.032459	1.138665
6.1	0.6442747	0.7116472	0.7776204	0.857797	0.9458702	1.032502	1.138318
6.2	0.6458446	0.7129685	0.7786981	0.8585827	0.9463438	1.032681	1.138156
6.3	0.647406	0.7142989	0.7798051	0.8594247	0.946907	1.032986	1.138168
6.4	0.6489526	0.7156324	0.7809346	0.8603161	0.9475526	1.033408	1.138347
6.5	0.6504794	0.7169635	0.7820812	0.8612512	0.9482746	1.033943	1.138685
6.6	0.6519814	0.7182871	0.7832397	0.8622246	0.9490674	1.034583	1.139175
6.7	0.6534535	0.7195979	0.7844045	0.8632306	0.9499248	1.035323	1.139813
6.8	0.6548906	0.7208908	0.7855704	0.8642635	0.9508411	1.036157	1.140591
6.9	0.6562881	0.7221609	0.7867322	0.865318	0.9518107	1.037078	1.141503
7	0.657641	0.723403	0.7878846	0.8663885	0.9528278	1.038082	1.142543
7.1	0.6589442	0.7246118	0.7890218	0.867469	0.9538858	1.03916	1.143705
7.2	0.6601934	0.7257826	0.7901393	0.8685545	0.9549797	1.040308	1.144982
7.3	0.6613867	0.7269136	0.7912351	0.8696433	0.956108	1.041524	1.146374
7.4	0.6625239	0.7280048	0.7923091	0.8707354	0.9572707	1.04281	1.147881
7.5	0.6636052	0.7290565	0.7933621	0.8718317	0.958469	1.044165	1.149505
7.6	0.6646323	0.7300705	0.7943958	0.8729343	0.9597053	1.045593	1.151251
7.7	0.6656067	0.7310488	0.7954127	0.8740457	0.9609826	1.047098	1.153121
7.8	0.6665306	0.7319934	0.7964147	0.8751685	0.9623037	1.048682	1.15512
7.9	0.6674058	0.7329065	0.7974043	0.8763051	0.9636713	1.050349	1.157251
8	0.6682361	0.733792	0.7983858	0.8774601	0.9650904	1.052105	1.159522
8.1	0.6690257	0.7346546	0.799364	0.8786388	0.966567	1.053955	1.161938
8.2	0.6697787	0.7354985	0.8003434	0.8798462	0.9681063	1.055906	1.164507
8.3	0.6704991	0.7363281	0.8013285	0.8810869	0.9697135	1.057962	1.167235
8.4	0.6711892	0.7371455	0.8023218	0.8823635	0.9713913	1.060128	1.170126
8.5	0.6718495	0.7379513	0.8033236	0.8836763	0.97314	1.062404	1.17318
8.6	0.6724792	0.7387443	0.8043323	0.8850234	0.9749573	1.064787	1.176395
8.7	0.673077	0.7395229	0.8053461	0.8864027	0.9768411	1.067275	1.179769

8.8	0.6736437	0.7402876	0.8063654	0.8878142	0.9787909	1.069867	1.183301
8.9	0.6741797	0.7410387	0.8073903	0.8892579	0.9808069	1.072563	1.186991
9	0.6746858	0.7417769	0.8084211	0.8907341	0.9828888	1.075364	1.19084
9.1	0.6751616	0.7425014	0.8094569	0.8922412	0.9850351	1.078266	1.194844
9.2	0.6756063	0.743211	0.8104961	0.8937775	0.9872433	1.081269	1.199002
9.3	0.6760188	0.7439044	0.8115371	0.8953407	0.989511	1.084368	1.20331
9.4	0.6763983	0.7445803	0.8125781	0.8969287	0.9918354	1.08756	1.207765
9.5	0.6767427	0.745236	0.813616	0.8985379	0.9942124	1.090842	1.212361
9.6	0.6770495	0.7458684	0.814647	0.9001635	0.9966363	1.094206	1.217092
9.7	0.6773161	0.7464747	0.8156676	0.9018013	0.9991021	1.097646	1.22195
9.8	0.6775405	0.7470518	0.8166744	0.9034472	1.001605	1.101158	1.226928
9.9	0.6777214	0.7475982	0.817665	0.9050979	1.00414	1.104735	1.232021
10	0.6778576	0.7481119	0.8186371	0.9067506	1.006705	1.108373	1.237224
10.1	0.6779471	0.7485906	0.8195878	0.9084017	1.009294	1.112068	1.242529
10.2	0.6779881	0.7490321	0.8205141	0.9100472	1.011903	1.115813	1.24793
10.3	0.6779781	0.7494329	0.8214121	0.9116824	1.014526	1.119601	1.253419
10.4	0.6779135	0.7497889	0.8222767	0.913301	1.017156	1.123423	1.258983
10.5	0.6777913	0.7500961	0.8231031	0.9148972	1.019784	1.12727	1.264613
10.6	0.6776089	0.7503516	0.8238876	0.9164664	1.022406	1.131135	1.2703
10.7	0.6773661	0.7505546	0.8246292	0.918007	1.025019	1.135017	1.27604
10.8	0.6770613	0.7507032	0.8253256	0.9195161	1.027619	1.138909	1.281829
10.9	0.6766933	0.7507958	0.825975	0.9209917	1.030205	1.14281	1.287662
11	0.6762609	0.7508315	0.826576	0.9224318	1.032773	1.146716	1.293534
11.1	0.6757634	0.7508088	0.8271269	0.9238344	1.035322	1.150624	1.299442
11.2	0.6751996	0.7507265	0.8276263	0.9251977	1.037848	1.154529	1.305381
11.3	0.6745684	0.7505832	0.8280725	0.9265193	1.040348	1.15843	1.311345
11.4	0.673867	0.750376	0.8284619	0.9277952	1.042818	1.162318	1.317327
11.5	0.6730915	0.7501001	0.8287895	0.9290193	1.04525	1.166186	1.323315
11.6	0.6722397	0.7497531	0.8290524	0.9301884	1.04764	1.170029	1.329304
11.7	0.6713094	0.7493326	0.8292477	0.9312987	1.049984	1.173841	1.335286
11.8	0.6702977	0.7488351	0.8293713	0.9323458	1.052276	1.177616	1.341254
11.9	0.6692013	0.7482567	0.8294189	0.9333243	1.054511	1.181346	1.347198
12	0.6680173	0.7475938	0.8293862	0.934229	1.056681	1.185024	1.353109
12.1	0.6667425	0.7468427	0.8292688	0.9350548	1.05878	1.188642	1.358976
12.2	0.6653736	0.7459995	0.8290619	0.9357957	1.060802	1.192191	1.364789
12.3	0.6639075	0.74506	0.8287608	0.936446	1.062738	1.195662	1.370535
12.4	0.6623397	0.7440192	0.8283592	0.9369979	1.06458	1.199043	1.376202
12.5	0.6606652	0.7428709	0.82785	0.9374428	1.066316	1.202322	1.38177
12.6	0.6588791	0.741609	0.8272259	0.9377716	1.067936	1.205485	1.387224
12.7	0.6569764	0.7402276	0.8264796	0.9379756	1.069429	1.208519	1.392546
12.8	0.6549541	0.7387225	0.825606	0.9380481	1.070785	1.211412	1.397722
12.9	0.6528122	0.7370933	0.8246041	0.9379872	1.072003	1.214159	1.402745
13	0.6505522	0.7353413	0.8234746	0.9377928	1.07308	1.216759	1.407608
13.1	0.6481769	0.7334688	0.8222197	0.9374667	1.074017	1.219211	1.412311
13.2	0.6456884	0.7314779	0.820841	0.9370099	1.074816	1.221513	1.416848
13.3	0.6430883	0.7293699	0.8193396	0.9364229	1.075474	1.223664	1.421217

13.4	0.6403785	0.7271463	0.8177167	0.9357063	1.075992	1.225662	1.425411
13.5	0.6375613	0.7248095	0.8159742	0.9348612	1.076371	1.227505	1.429428
13.6	0.6346392	0.7223617	0.8141142	0.9338896	1.07661	1.229194	1.433264
13.7	0.6316147	0.7198051	0.8121386	0.9327925	1.07671	1.230726	1.436917
13.8	0.6284897	0.7171416	0.8100489	0.931571	1.076671	1.232101	1.440381
13.9	0.6252669	0.7143736	0.8078473	0.9302265	1.076494	1.233317	1.443653
14	0.6219498	0.7115045	0.8055369	0.9287619	1.07618	1.234375	1.446731
14.1	0.6185417	0.7085376	0.8031207	0.9271798	1.075732	1.235277	1.449614
14.2	0.6150467	0.7054771	0.8006033	0.9254843	1.075153	1.236023	1.452301
14.3	0.6114693	0.7023273	0.7979887	0.9236795	1.074446	1.236618	1.454795
14.4	0.6078137	0.6990929	0.7952816	0.92177	1.073616	1.237064	1.457098
14.5	0.604085	0.6957789	0.7924872	0.9197608	1.072668	1.237366	1.459213
14.6	0.6002884	0.6923907	0.789611	0.9176577	1.071607	1.23753	1.461144
14.7	0.5964291	0.6889338	0.7866586	0.9154664	1.070439	1.237561	1.462896
14.8	0.5925122	0.6854134	0.7836356	0.9131924	1.06917	1.237464	1.464474
14.9	0.5885428	0.6818349	0.7805473	0.9108413	1.067805	1.237245	1.465882
15	0.5845259	0.6782035	0.7773993	0.908419	1.06635	1.236909	1.467127
15.1	0.5804664	0.6745245	0.774197	0.9059309	1.064811	1.236462	1.468214
15.2	0.5763696	0.6708032	0.7709463	0.9033833	1.063194	1.235911	1.469149
15.3	0.5722407	0.6670454	0.7676527	0.900782	1.061505	1.235262	1.469938
15.4	0.5680845	0.6632561	0.764322	0.8981331	1.05975	1.23452	1.470588
15.5	0.5639067	0.6594414	0.7609604	0.8954433	1.057938	1.233695	1.471107
15.6	0.5597169	0.6556112	0.7575786	0.8927244	1.056081	1.232803	1.471518
15.7	0.5555238	0.6517746	0.7541862	0.8899872	1.054192	1.23186	1.471843
15.8	0.5513322	0.6479365	0.7507882	0.8872374	1.052279	1.230875	1.472094
15.9	0.5471467	0.6441016	0.7473899	0.8844813	1.05035	1.229858	1.472284
16	0.5429721	0.6402749	0.7439967	0.8817247	1.04841	1.228817	1.472425
16.1	0.5388123	0.6364605	0.7406129	0.8789725	1.046467	1.227761	1.47253
16.2	0.5346712	0.6326622	0.7372422	0.8762292	1.044526	1.226696	1.47261
16.3	0.5305529	0.6288839	0.7338889	0.8734993	1.042593	1.225632	1.472674
16.4	0.5264598	0.6251281	0.7305558	0.8707862	1.040671	1.224572	1.472732
16.5	0.5223921	0.6213956	0.7272437	0.8680911	1.038763	1.223519	1.472785
16.6	0.5183499	0.6176867	0.7239533	0.8654151	1.03687	1.222475	1.472835
16.7	0.5143335	0.6140019	0.7206857	0.8627595	1.034993	1.221441	1.472885
16.8	0.5103435	0.6103424	0.7174422	0.8601261	1.033134	1.22042	1.472936
16.9	0.5063805	0.6067091	0.7142242	0.8575169	1.031297	1.219415	1.472991
17	0.502445	0.603103	0.7110331	0.8549337	1.029483	1.218428	1.473053
17.1	0.4985373	0.5995249	0.7078702	0.8523781	1.027695	1.21746	1.473125
17.2	0.4946578	0.5959753	0.7047362	0.8498511	1.025933	1.216514	1.473205
17.3	0.4908053	0.5924533	0.7016302	0.8473518	1.024196	1.215588	1.473294
17.4	0.486978	0.5889568	0.69855	0.8448777	1.022481	1.214678	1.473385
17.5	0.4831737	0.5854836	0.6954931	0.8424258	1.020785	1.213779	1.473473
17.6	0.4793895	0.5820303	0.6924558	0.8399917	1.019102	1.212885	1.473549
17.7	0.4756228	0.5785937	0.6894341	0.8375708	1.017427	1.211989	1.473606
17.8	0.4718708	0.5751708	0.6864247	0.835159	1.015754	1.211085	1.473633
17.9	0.4681311	0.571759	0.6834245	0.8327524	1.014078	1.210167	1.473625

18	0.4644014	0.5683552	0.68043	0.8303469	1.012396	1.209228	1.473572
18.1	0.4606797	0.5649574	0.6774389	0.8279397	1.010702	1.208263	1.473469
18.167	0.4581907	0.5626845	0.6754369	0.8263261	1.009561	1.207603	1.473371

Supplemental Data Table 2: Numerical centile values for plasma creatinine at discrete ages.

Age (year)	Plasma creatinine concentration (µmol/L)						
	2.5th	10th	25th	50th	75th	90th	97.5th
0.05	20.39485	22.73694	25.0653	27.93971	31.15173	34.36477	38.35862
0.1	20.4914	22.85315	25.19565	28.08009	31.29369	34.49834	38.46825
0.2	20.6849	23.08624	25.457	28.3613	31.57795	34.76615	38.68958
0.3	20.87967	23.32091	25.71992	28.64389	31.86358	35.03593	38.91504
0.4	21.0766	23.55806	25.98532	28.92888	32.15176	35.30907	39.14625
0.5	21.27639	23.79837	26.25391	29.21702	32.44336	35.58656	39.38427
0.6	21.47948	24.04226	26.52609	29.50876	32.7389	35.86898	39.62972
0.7	21.68613	24.2899	26.80201	29.80424	33.03854	36.15651	39.88273
0.8	21.89625	24.54114	27.08148	30.10323	33.34201	36.44883	40.14284
0.9	22.10941	24.79547	27.36389	30.40506	33.64859	36.74512	40.409
1	22.32508	25.05229	27.64861	30.70904	33.95747	37.04445	40.68007
1.1	22.54292	25.31118	27.93517	31.01463	34.26808	37.34616	40.95522
1.2	22.76263	25.57179	28.22316	31.3214	34.57995	37.64973	41.2338
1.3	22.984	25.83384	28.51226	31.62901	34.89269	37.95473	41.51524
1.4	23.20685	26.09712	28.80227	31.93721	35.20607	38.26085	41.79917
1.5	23.43114	26.36157	29.09307	32.24592	35.51996	38.56798	42.08537
1.6	23.65686	26.62713	29.38463	32.55509	35.83434	38.87606	42.37371
1.7	23.88396	26.89375	29.67689	32.86465	36.14913	39.18499	42.66405
1.8	24.1124	27.16135	29.96975	33.17454	36.46426	39.49468	42.95622
1.9	24.3421	27.42984	30.26312	33.48464	36.77962	39.80502	43.25005
2	24.57303	27.69915	30.55692	33.79489	37.09514	40.11589	43.54537
2.1	24.80511	27.9692	30.85108	34.10519	37.41073	40.4272	43.84206
2.2	25.03828	28.23989	31.14548	34.41547	37.7263	40.73885	44.13995
2.3	25.27247	28.51115	31.44005	34.72564	38.04178	41.05075	44.43891
2.4	25.50762	28.7829	31.73471	35.03562	38.35709	41.36281	44.73882
2.5	25.74366	29.05505	32.02938	35.34534	38.67214	41.67492	45.03953
2.6	25.98052	29.32752	32.32397	35.65471	38.98687	41.98701	45.34093
2.7	26.21815	29.60023	32.6184	35.96368	39.30119	42.299	45.64289
2.8	26.45646	29.87311	32.91261	36.27216	39.61504	42.61079	45.94533
2.9	26.69541	30.14609	33.20653	36.5801	39.92838	42.92233	46.24813
3	26.93492	30.4191	33.50009	36.88744	40.24112	43.23355	46.5512
3.1	27.17492	30.69205	33.7932	37.19411	40.55321	43.54437	46.85444
3.2	27.41533	30.96486	34.0858	37.50004	40.86458	43.85472	47.15776
3.3	27.65609	31.23747	34.37783	37.80517	41.17517	44.16454	47.46106
3.4	27.89709	31.50978	34.66918	38.10943	41.48492	44.47374	47.76426
3.5	28.13823	31.78165	34.95975	38.41268	41.79367	44.78217	48.06718
3.6	28.37935	32.05295	35.24936	38.71477	42.10129	45.08967	48.3696
3.7	28.62032	32.32351	35.53786	39.01554	42.40758	45.39605	48.67134
3.8	28.86097	32.59316	35.82508	39.31481	42.71238	45.70111	48.97216

3.9	29.10115	32.86174	36.11086	39.61241	43.0155	46.00466	49.27186
4	29.34067	33.12904	36.39498	39.90813	43.31672	46.30648	49.57018
4.1	29.57933	33.39486	36.67722	40.20173	43.61578	46.60627	49.8668
4.2	29.81691	33.65894	36.95733	40.49296	43.91244	46.90378	50.16144
4.3	30.0532	33.92109	37.23509	40.78159	44.20643	47.19872	50.4538
4.4	30.28799	34.18105	37.51025	41.06735	44.4975	47.49083	50.74358
4.5	30.52102	34.43856	37.78254	41.34996	44.78531	47.77976	51.03041
4.6	30.75201	34.69332	38.05162	41.62907	45.06953	48.06514	51.31389
4.7	30.98072	34.94506	38.31723	41.90441	45.34985	48.34666	51.59368
4.8	31.20691	35.19353	38.57911	42.17568	45.62597	48.62399	51.86945
4.9	31.43034	35.43847	38.83698	42.44263	45.89763	48.89687	52.1409
5	31.65077	35.67964	39.0906	42.70499	46.16454	49.16499	52.40771
5.1	31.868	35.91681	39.33972	42.96251	46.42643	49.42807	52.66959
5.2	32.08189	36.14985	39.58423	43.21507	46.68321	49.68601	52.92642
5.3	32.29241	36.37874	39.82411	43.46268	46.93487	49.93882	53.17823
5.4	32.4995	36.60342	40.05932	43.70529	47.1814	50.18649	53.425
5.5	32.70301	36.82376	40.28973	43.9428	47.42268	50.42891	53.66662
5.6	32.90283	37.03965	40.51524	44.1751	47.65862	50.66598	53.903
5.7	33.09882	37.25096	40.73571	44.40207	47.8891	50.89758	54.13402
5.8	33.29087	37.45757	40.95105	44.62362	48.11403	51.12365	54.35961
5.9	33.47887	37.65939	41.16118	44.83968	48.33334	51.3441	54.57969
6	33.66272	37.85633	41.36602	45.05017	48.54699	51.55888	54.79422
6.1	33.84237	38.04837	41.56553	45.25508	48.75494	51.76799	55.00317
6.2	34.01777	38.23545	41.7597	45.4544	48.9572	51.97142	55.20657
6.3	34.1889	38.41759	41.94856	45.64816	49.15383	52.16924	55.40447
6.4	34.35581	38.59487	42.13219	45.83649	49.34495	52.3616	55.59704
6.5	34.51857	38.76738	42.31073	46.01954	49.53076	52.5487	55.78451
6.6	34.67726	38.93523	42.48431	46.19748	49.71143	52.73074	55.96708
6.7	34.83188	39.09848	42.65301	46.3704	49.8871	52.90786	56.14492
6.8	34.98246	39.25718	42.81691	46.53841	50.05786	53.08019	56.31816
6.9	35.12901	39.41134	42.97605	46.70157	50.22381	53.24783	56.48692
7	35.27148	39.56097	43.13044	46.85991	50.38499	53.41081	56.65123
7.1	35.40985	39.70605	43.28009	47.01342	50.54141	53.56917	56.81115
7.2	35.54408	39.84657	43.42498	47.16214	50.6931	53.72295	56.96672
7.3	35.67423	39.9826	43.56522	47.30618	50.84019	53.87229	57.1181
7.4	35.80042	40.11432	43.70103	47.44578	50.98297	54.01748	57.26558
7.5	35.92285	40.24196	43.83266	47.58124	51.12175	54.15888	57.40956
7.6	36.04173	40.3658	43.96042	47.71291	51.2569	54.29689	57.55047
7.7	36.15725	40.48606	44.08459	47.84108	51.38878	54.43187	57.68869
7.8	36.26956	40.60295	44.20539	47.96602	51.51765	54.56411	57.82453
7.9	36.37878	40.71662	44.32302	48.08796	51.64378	54.6939	57.9583
8	36.48505	40.82724	44.43766	48.2071	51.7674	54.82149	58.09026
8.1	36.58851	40.935	44.54954	48.32369	51.88875	54.94714	58.2207
8.2	36.68936	41.04014	44.65892	48.43803	52.00819	55.07123	58.35001
8.3	36.78783	41.14293	44.7661	48.55045	52.12606	55.19413	58.4786
8.4	36.88409	41.24358	44.87136	48.66125	52.24271	55.3162	58.60684

8.5	36.97829	41.3423	44.9749	48.77069	52.35841	55.43774	58.73505
8.6	37.07056	41.43925	45.07692	48.87897	52.47338	55.55898	58.8635
8.7	37.161	41.53456	45.17758	48.98629	52.58785	55.68018	58.99245
8.8	37.2497	41.62836	45.27705	49.09283	52.70202	55.80156	59.12214
8.9	37.33676	41.72079	45.37547	49.19876	52.8161	55.92332	59.2528
9	37.42227	41.81197	45.473	49.30428	52.93027	56.0457	59.38466
9.1	37.50633	41.90203	45.56979	49.40955	53.04474	56.16888	59.51795
9.2	37.58903	41.99109	45.66598	49.51472	53.15967	56.29308	59.65288
9.3	37.67046	42.07927	45.76171	49.61997	53.27526	56.41847	59.78965
9.4	37.75071	42.16668	45.85712	49.72544	53.39166	56.54525	59.92847
9.5	37.82987	42.25344	45.95235	49.83131	53.50907	56.67361	60.06955
9.6	37.90805	42.33969	46.04753	49.9377	53.62764	56.80373	60.21306
9.7	37.98532	42.4255	46.14277	50.04474	53.74748	56.93572	60.35915
9.8	38.06175	42.51096	46.23814	50.15252	53.86871	57.06971	60.50794
9.9	38.13745	42.59619	46.33381	50.26122	53.99152	57.20589	60.65965
10	38.21257	42.68136	46.42994	50.37101	54.1161	57.34449	60.8145
10.1	38.28722	42.76661	46.52669	50.48208	54.24265	57.4857	60.97273
10.2	38.36152	42.85207	46.62421	50.59459	54.37134	57.62972	61.13453
10.3	38.43558	42.93787	46.72263	50.70867	54.50233	57.77672	61.30009
10.4	38.5095	43.02411	46.82206	50.82444	54.63574	57.92683	61.46955
10.5	38.58338	43.11087	46.9226	50.94203	54.7717	58.08017	61.64307
10.6	38.6573	43.19827	47.02436	51.06154	54.91033	58.2369	61.82079
10.7	38.73135	43.28639	47.12743	51.18306	55.05172	58.3971	62.0028
10.8	38.80561	43.37529	47.23186	51.30663	55.19592	58.56082	62.18916
10.9	38.88017	43.46506	47.33773	51.43235	55.34303	58.72816	62.37998
11	38.95515	43.55581	47.44515	51.56033	55.49314	58.89924	62.57539
11.1	39.03065	43.64764	47.55423	51.69067	55.64638	59.07417	62.77549
11.2	39.1068	43.74066	47.66506	51.82346	55.80283	59.25304	62.98042
11.3	39.18369	43.83497	47.77775	51.9588	55.96261	59.43598	63.19027
11.4	39.26146	43.93068	47.89239	52.0968	56.12582	59.62309	63.40517
11.5	39.34017	44.02785	48.00905	52.23751	56.29249	59.81441	63.62518
11.6	39.41989	44.12651	48.12773	52.38092	56.46262	60.00993	63.85026
11.7	39.50069	44.22673	48.24848	52.52706	56.63624	60.20967	64.08045
11.8	39.58264	44.32853	48.3713	52.67593	56.81332	60.41361	64.31573
11.9	39.66576	44.4319	48.49617	52.82748	56.99383	60.6217	64.55604
12	39.75011	44.53687	48.62309	52.98169	57.1777	60.83389	64.80132
12.1	39.83567	44.64338	48.75198	53.13847	57.36485	61.05005	65.05146
12.2	39.92245	44.75141	48.88279	53.29775	57.55518	61.27012	65.30637
12.3	40.01046	44.86095	49.0155	53.45948	57.74865	61.49402	65.56601
12.4	40.09974	44.97201	49.15009	53.62363	57.94522	61.72172	65.83031
12.5	40.19034	45.08461	49.28656	53.7902	58.14486	61.95319	66.09927
12.6	40.28231	45.19875	49.4249	53.95915	58.34753	62.1884	66.37285
12.7	40.3757	45.31446	49.56511	54.13046	58.55321	62.4273	66.65102
12.8	40.47049	45.4317	49.70712	54.30404	58.7618	62.6698	66.93369
12.9	40.56661	45.55034	49.85077	54.47972	58.97309	62.91569	67.22062
13	40.66397	45.67027	49.99594	54.65733	59.18691	63.16475	67.51159

13.1	40.76251	45.79139	50.14249	54.8367	59.40305	63.41677	67.80637
13.2	40.86213	45.91357	50.29024	55.01765	59.62129	63.67153	68.10474
13.3	40.96279	46.03672	50.43911	55.20004	59.8415	63.92885	68.40648
13.4	41.06448	46.16082	50.58902	55.38378	60.06355	64.18862	68.71149
13.5	41.16722	46.28587	50.73998	55.56886	60.28743	64.45081	69.01974
13.6	41.27117	46.41201	50.89211	55.75541	60.51327	64.71555	69.3314
13.7	41.3765	46.5394	51.04558	55.9436	60.74126	64.98306	69.64669
13.8	41.48337	46.66821	51.20054	56.13358	60.97155	65.25353	69.96585
13.9	41.59193	46.79856	51.35712	56.32549	61.20432	65.52714	70.28908
14	41.70229	46.93058	51.51546	56.51947	61.4397	65.80405	70.61657
14.1	41.81454	47.06437	51.67566	56.71565	61.67784	66.08439	70.94848
14.2	41.92873	47.19999	51.83778	56.91407	61.91878	66.36824	71.28489
14.3	42.04479	47.33735	52.00172	57.11463	62.16241	66.65548	71.62569
14.4	42.1626	47.47633	52.16735	57.31718	62.40857	66.94595	71.9707
14.5	42.28205	47.61681	52.33455	57.52158	62.6571	67.23945	72.31975
14.6	42.40304	47.75868	52.50316	57.72766	62.90783	67.53581	72.67263
14.7	42.52547	47.90183	52.6731	57.93531	63.1606	67.83488	73.02918
14.8	42.64925	48.04615	52.8442	58.14436	63.41526	68.13648	73.38922
14.9	42.77427	48.19153	53.01637	58.35469	63.67165	68.44044	73.75256
15	42.90042	48.33786	53.18948	58.56615	63.92961	68.74657	74.11902
15.1	43.0276	48.48501	53.36339	58.77859	64.18896	69.05469	74.4884
15.2	43.15562	48.63281	53.53791	58.99179	64.44947	69.36455	74.8604
15.3	43.2843	48.78107	53.71283	59.20554	64.7109	69.67587	75.23476
15.4	43.41343	48.92958	53.88795	59.41959	64.97298	69.98838	75.61115
15.5	43.54281	49.07814	54.06305	59.63371	65.23544	70.30175	75.9892
15.6	43.67225	49.22654	54.23788	59.84763	65.49796	70.61565	76.36856
15.7	43.80162	49.37463	54.4123	60.06116	65.76035	70.92986	76.74898
15.8	43.93089	49.52239	54.58625	60.27427	66.02254	71.24429	77.13036
15.9	44.06003	49.6698	54.75974	60.4869	66.28449	71.55888	77.51262
16	44.18901	49.81682	54.9327	60.69902	66.54613	71.87356	77.89568
16.1	44.31775	49.96336	55.10506	60.91051	66.80733	72.18818	78.27937
16.2	44.44627	50.10948	55.27684	61.12143	67.06815	72.5028	78.66377
16.3	44.57464	50.25522	55.44813	61.33183	67.32865	72.81752	79.049
16.4	44.70287	50.4006	55.61893	61.54176	67.58889	73.13239	79.43513
16.5	44.831	50.54569	55.78931	61.75127	67.84893	73.44746	79.82226
16.6	44.9591	50.69055	55.95935	61.96046	68.10888	73.76289	80.2105
16.7	45.08723	50.83526	56.12915	62.16943	68.36884	74.07878	80.60002
16.8	45.21541	50.97985	56.29873	62.37821	68.62886	74.39518	80.99087
16.9	45.34364	51.12434	56.46811	62.58683	68.88896	74.71211	81.38309
17	45.47194	51.26874	56.63732	62.79531	69.14919	75.02963	81.77674
17.1	45.60031	51.41307	56.80637	63.00368	69.40954	75.34776	82.17184
17.2	45.72876	51.55733	56.97528	63.21194	69.67005	75.66651	82.56846
17.3	45.85735	51.7016	57.14413	63.4202	69.93082	75.98602	82.96673
17.4	45.98612	51.84593	57.31296	63.62849	70.19191	76.30637	83.36676
17.5	46.11512	51.99036	57.48183	63.8369	70.45341	76.62764	83.76868
17.6	46.24439	52.13493	57.65078	64.04544	70.71534	76.94989	84.17258

17.7	46.37393	52.27962	57.81978	64.2541	70.9777	77.27312	84.57848
17.8	46.50373	52.42443	57.98882	64.46286	71.24046	77.59733	84.98639
17.9	46.6338	52.56934	58.15786	64.67167	71.50357	77.92248	85.39632
18	46.7641	52.7143	58.32685	64.88049	71.76701	78.24853	85.80828
18.1	46.89464	52.85931	58.49579	65.08928	72.03074	78.57549	86.22227
18.17	46.98615	52.96083	58.614	65.23542	72.21552	78.80489	86.5133

Research Article

Adolopment of Recommendations for Standardized Reporting of Protein Electrophoresis in Pakistan

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Keywords
Protein Electrophoresis, guidelines, Pakistan, reporting

Abstract

Introduction
The standardization of reporting in clinical laboratories, particularly regarding Serum Protein Electrophoresis (SPEP) and Urine Protein Electrophoresis (UPEP), is crucial for effective communication of findings to clinicians and optimal patient management. However, in countries like Pakistan with limited healthcare resources and a prevalent self-payment model, challenges arise in achieving standardized reporting practices. This manuscript addresses the need for standardized guidelines for protein electrophoresis reporting in Pakistan, aiming to enhance laboratory practices and patient care.

Methods
This study was conducted at the Aga Khan University Hospital (AKUH), Pakistan. A team consisting of five Consultant Chemical Pathologists and two senior technologists, led by the Section Head of Chemical Pathology at AKU, used a Modified Delphi Methodology to achieve consensus on the developed framework. Consensus was defined as agreement by at least six out of the seven experts (85.71%). The source guideline for this process was the Recommendations for Standardized Reporting of Protein Electrophoresis from Australia and New Zealand.

Results
Consultant Chemical Pathologists reviewed the original and modified recommendations, resulting in a framework of ten sub-sections and 65 recommendations. Through a series of four meetings, including a diverse team of experts, the recommendations were systematically critiqued and reviewed. After detailed deliberations, 54 recommendations were finalized by consensus. The final document was further reviewed by CCBP staff and additional consultants from different institutions in Pakistan to ensure unbiased and comprehensive expert input.

Discussion

The developed guidelines offer a framework for consistent and comprehensive reporting of PEP results, addressing variations in practices among clinical laboratories in Pakistan. Key modifications to the recommendations reflect a pragmatic approach to navigating resource constraints, ensuring that laboratory reports remain informative and actionable for clinicians. By prioritizing clinical relevance and practicality, the guidelines aim to enhance diagnostic accuracy and facilitate appropriate clinical management decisions.

Conclusion

The standardized reporting guidelines for SPEP and UPEP represent a significant milestone in optimizing laboratory practices and improving patient care in Pakistan. Moving forward, continued monitoring and adaptation of the guidelines will be essential to ensure their sustained relevance and effectiveness in meeting the evolving needs of the healthcare system. Embracing a commitment to excellence in laboratory practices holds promise for advancing healthcare quality and accessibility in low-resource settings globally.

Introduction

The fundamental purpose of conducting Serum Protein Electrophoresis (SPEP) and Urine Protein Electrophoresis (UPEP) is to identify monoclonal immunoglobulins associated with plasma cell dyscrasias and lymphoproliferative disorders. Ensuring effective communication of laboratory findings to clinicians is of utmost importance in guiding patient management. However, achieving this goal requires a thorough understanding of the requisites of a protein electrophoresis report. The standardization of reporting in Pakistan encounters challenges stemming from the absence of a national health insurance system and the prevalent self-payment model for medical care including laboratory investigations [1]. Consequently, immunofixation and electrophoresis reports are frequently issued in isolation, rather than as paired assessments, primarily due to practice of a cost-effective model by physicians for patient care [2].

The delivery of a comprehensible laboratory report is vital in aiding clinicians in patient management. Clinicians are chiefly concerned with the presence, types, and concentrations of paraproteins. Having access to a cumulative report is imperative for monitoring plasma cell dyscrasias [3].

Several notable findings emerge from Protein Electrophoresis (PEP), such as increased alpha-1 and alpha-2 globulins indicating acute phase response, decreased alpha-1 globulins suggestive of alpha-1 antitrypsin deficiency, increased beta-1 region indicative of elevated transferrin and iron deficiency, decreased gamma globulins, and a diverse gamma globulin increase reflecting inflammation, infection, autoimmune disorders, or liver diseases [4].

In Pakistan, where healthcare resources are limited and financial constraints exist, the need for standardized reporting of tests like SPEP and immunofixation electrophoresis (IFE) on which diagnoses are made becomes even more significant. Efforts towards standardization must consider the local healthcare infrastructure and availability of resources in clinical laboratories. This involves improving and following the best laboratory practices as per available guidelines and literature and aligning with available resources. This will ensure that laboratory professionals and clinicians can make well-informed decisions based on the information provided [5].

Notwithstanding the presence of established clinical guidelines pertaining to plasma cell dyscrasias, there is a notable lack of emphasis on the laboratory aspects of PEP. Notably, systematic reporting standards and recommendations are scarce in literature. A review of the literature revealed that there are currently no standardized guidelines or recommendations being followed in Pakistan aimed at analytical performance and reporting of PEP.

With the above explained problem statement in perspective, a survey was conducted to analyze the clinical laboratory practices, the method of quantification of paraprotein concentrations by PEP, and interpretation provided by Consultant Chemical Pathologists performing PEP in Pakistan. The findings highlighted variations in practices of PEP, resulting in variable and inconsistent reporting, affecting patient care.

A literature review detailed in the methods section revealed recommendations from Australia and New Zealand, Canada, and Malaysia [6-9]. Given that Australia and New Zealand, like Pakistan, are Commonwealth countries with similar clinical practices, we preferred to tailor our recommendations according to their developed guidelines. In contrast, Canada's healthcare system operates differently, primarily based on public insurance, with approximately 70% of health expenditures financed through general tax revenues [10]. On the other hand, Malaysia has adopted guidelines from Australia and New Zealand, and Canada [9].

However, it is crucial to recognize that Pakistan's healthcare system primarily relies on out-of-pocket payments [11], unlike the healthcare systems in Australia and New Zealand. Australia's health system responsibilities are broadly shared between the Australian government and state and territory governments, involving funding, operating, managing, and regulating the health system [12]. Similarly, New Zealand's healthcare system is mostly tax-funded [13].

Consequently, there is immense need for local recommendations to be developed, with appropriate context-specific modifications. The development of standardized reporting guidelines for PEP stands to offer significant advantages to pathologists nationwide, thereby facilitating substantial benefits across the spectrum of pre-analytical, analytical, and post-analytical processes. Providing relevant information about response criteria and paraprotein presence while adapting to the local healthcare dynamics can significantly contribute to improved patient management and outcomes.

Methods

Setting

This study was conducted at the Section of Chemical Pathology, Department of Pathology and Laboratory Medicine at the Aga Khan University Hospital (AKU), Pakistan in collaboration with the expertise of the Clinical and Translational Research Incubator (CITRIC) Center for Clinical Best Practices (CCBP), at AKU.

Study team

The study team was comprised of the five Chemical Pathology faculties and two senior technologists led by the Section Head of Chemical Pathology at AKU. Modified Delphi Methodology [14] was adopted to take consensus on the developed framework. Consensus was achieved when at least six out of the seven experts (85.71%) involved in the decision-making process agreed on the proposed adaptations or modifications to the guidelines.

Source guideline selection

The source guideline is the single, original, "parent" guidelines that undergoes the ADOLOPMENT process in the development of a local documents.

A literature review was conducted using the search string: ("protein s"[All Fields] OR "proteinous"[All Fields] OR "proteins"[MeSH Terms] OR "proteins"[All Fields] OR "protein"[All Fields]) AND ("electrophoresed"[All Fields] OR "electrophoresing"[All Fields] OR "electrophoresis"[MeSH Terms] OR "electrophoresis"[All Fields] OR "electrophorese"[All Fields] OR "electrophoreses"[All Fields]) AND ("reference standards"[MeSH Terms] OR ("reference"[All Fields] AND "standards"[All Fields]) OR "reference standards"[All Fields] OR "standardization"[All Fields] OR "standard"[All Fields] OR "standard s"[All Fields] OR "standardisation"[All Fields] OR "standardisations"[All Fields] OR "standardise"[All Fields] OR "standardised"[All Fields] OR "standardises"[All Fields] OR "standardising"[All Fields] OR "standardization s"[All Fields] OR "standardizations"[All Fields] OR "standardize"[All Fields] OR "standardized"[All Fields] OR "standardizes"[All Fields] OR "standardizing"[All Fields] OR "standards"[MeSH Subheading] OR "standards"[All Fields]) AND ("reportable"[All Fields] OR "reporting"[All Fields] OR "reportings"[All Fields] OR "research report"[MeSH Terms] OR ("research"[All Fields] AND "report"[All Fields]) OR "research report"[All Fields] OR "report"[All Fields] OR "reported"[All Fields] OR "reports"[All Fields]) AND ("guideline"[Publication Type] OR "guidelines as topic"[MeSH Terms] OR "guidelines"[All Fields]) on PubMed, Medscape, and Google Scholar.

Recommendations for standardized reporting of protein electrophoresis in Australia and New Zealand were selected due to its comprehensive set of recommendations, integrated approach to management, and high-quality synthesis of available evidence [6].

Results

Framework

A Consultant Chemical Pathologist thoroughly reviewed the recommendations and their following modifications by the original group published in 2012 and 2019 respectively [6, 7]. A tabulated framework consisting of ten sub sections and a total of 65 recommendations was formulated. Three options- adopt, adapt and remove- were provided with each recommendation for expert review.

Expert panel review

In the first phase, the recommendations were reviewed by two Chemical Pathology consultants (SA and IS) and their responses were recorded against each criterion and a skeleton was built for the team as shown in Figure 1.

In the second phase, three more subsequent meetings were conducted (in the presence of the above-mentioned team), in which the recommendations and responses from the first phase were critiqued and reviewed systematically.

The second meeting was convened in the subsequent week, which included a broader team consisting of SA, IS, LJ, HM, AHK, and SK. This team focused on reviewing guidelines numbered 1 to 25. Following this, another meeting took place four days later, due to time constraints of the consultant pathologists, concentrating on guidelines 26 to 45.

In the fourth meeting, two days after the third, the team expanded further with the addition of RK and SK, two senior technologists. Together, this team of experts reviewed guidelines 46 to 65. The cumulative efforts of this group of experts aimed at ensuring the guidelines were tailored to meet the specific needs and standards relevant to the Pakistani context.

The final outcome generated through modified Delphi process was in the form of a single selection from multiple response options based on consensus and reasoning from experts. Out of a total of 65, 15 recommendations underwent minor changes in the response criteria from the first phase, and 3 guidelines were merged into a single recommendation. A total of 10 were excluded because they were not suitable for the Pakistani health care setup.

Final recommendation revisions and synthesis

Following the 2 phases of detailed deliberations, a total of 54 recommendations were finalized after consensus as depicted in Table 1. The CCBP staff conducted a meeting with the expert panel's sub-team to review the final unanimous consensus and to look for the need for any revisions. The consensus document was presented to the team for final assessment, in addition to 4 consultants, M.D.K, Q.A.K, S.I, G.A, from different institutions across Pakistan to minimize bias and broaden our level of expertise.

Figure 1: Process of adolopment of recommendations.

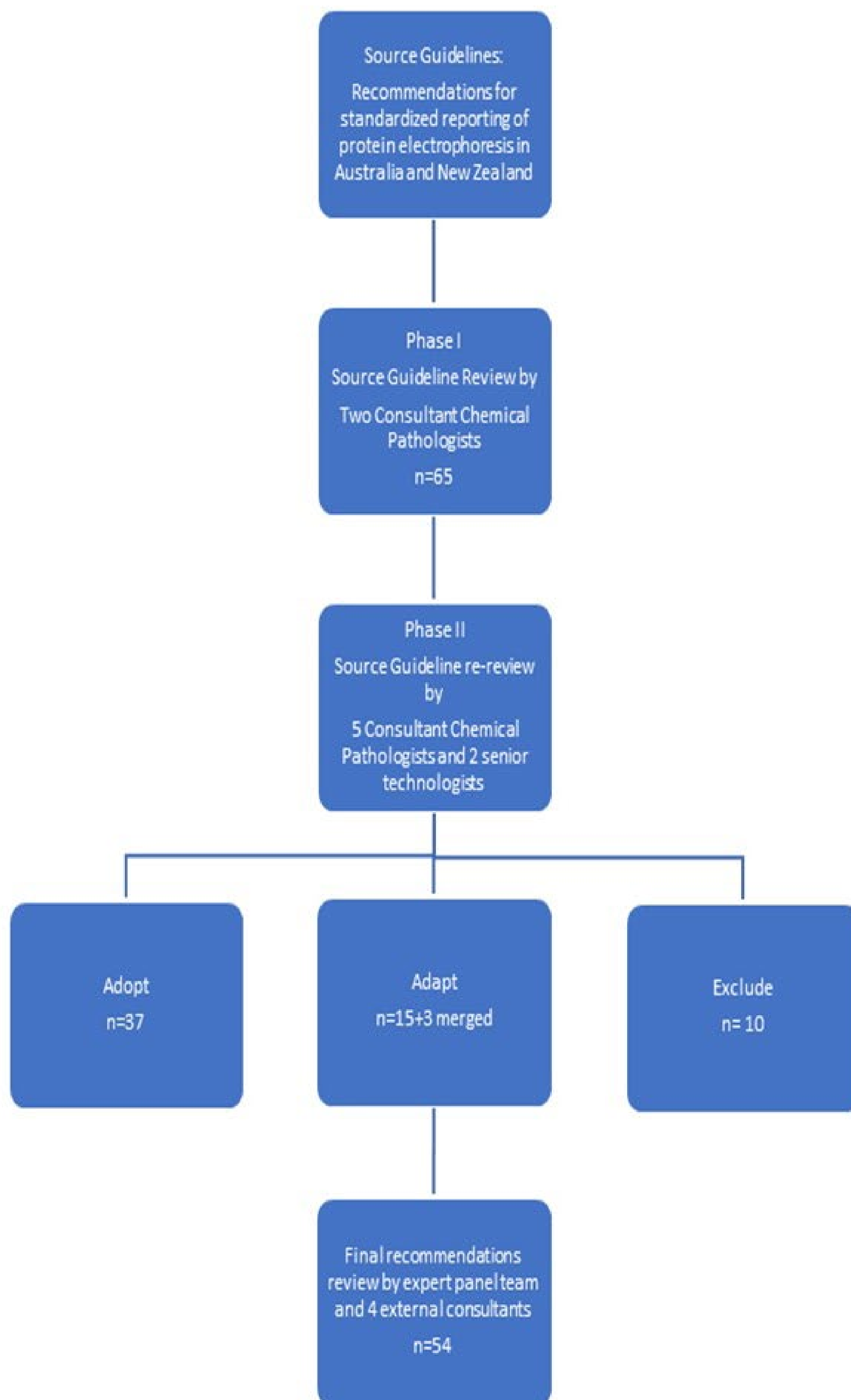

Table 1: Guidelines for the Detection, Quantification, and Reporting of Paraproteins in Serum and Urine: Standardized Nomenclature, Methodology, and Interpretative Commentary.

S No.	Recommendations
Nomenclature:	
1	The monoclonal component in serum is referred to as a Paraprotein (preferable) or Monoclonal immunoglobulin e.g. IgG kappa paraprotein or monoclonal IgG kappa.
2	The term Monoclonal free light chains is preferred to Bence Jones protein (BJP) when referring to urinary monoclonal free light chains (FLC).
3	The monoclonal component in urine is referred to generally as paraprotein or specifically as BJP or monoclonal FLCs.
Detection system for protein electrophoresis:	
4	The electrophoretic system preferably should be of high resolution and be able to detect small monoclonal bands that may co-migrate with normal proteins particularly in the beta region. However, low-resolution electrophoresis on cellulose acetate is acceptable for protein electrophoresis in case of non-availability of high-resolution system.
5	Clinicians should be encouraged to monitor the paraprotein concentration in individual patients using the same method (used by the same laboratory or laboratory network), hence ensuring analysts have access to the cumulative reports of the paraprotein delineation on the densitometric/capillary zone electrophoresis (CZE) scan
6	Isoelectric focusing (IEF) may occasionally be required in certain situations such as when examining serum samples of patients who are post-stem cell transplantation. For example, IEF may help to ascertain, if a low-concentration band detected on immunofixation electrophoresis (IFE) is the same as the paraprotein originally found in the patient's serum samples or is a new monoclonal protein, or if the band(s) on SPEP are oligoclonal. If a laboratory does not perform IEF, serum samples of patients should be referred to a reference laboratory in problematic cases.
Serum protein and albumin quantification:	
7	Total protein and albumin quantification as determined by an automated analyzer be available for assessment of the protein electrophoresis
8	Serum albumin quantification by bromocresol purple (BCP) or CZE is preferable to quantification by bromocresol green (BCG) although all are acceptable
9	Providing the same albumin result on the SPEP report as on the General Chemistry report is preferable but may not be possible depending upon the available Laboratory Information System
10	Total protein and albumin should be quantified in g/L to the nearest whole number
Quantitative reporting of SPEP fractions:	
11	The minimal quantitative fields to be reported are total protein and albumin; and, if present, the paraprotein(s)
12	The quantitative reporting of all SPEP fractions is optional
13	Protein fractions should be quantified in g/L to the nearest whole number
14	Laboratories should determine their own reference intervals or validate published reference intervals
15	Paraprotein(s) should be consistently reported in the same quantitative field to facilitate long-term cumulative review of the progress of a patient's disease and avoid misinterpretation of results
Serum para protein quantification:	
16	Paraproteins in the gamma region should be quantified by densitometric or CZE measurement in g/L rounded to the nearest whole number
17	Paraproteins of <1 g/L visible on SPEP or CZE cannot be quantified reliably especially if there is a polyclonal gamma globulin background and should be referred to as '<1 g/L' or 'trace' with comments such as 'small band cannot be quantified reliably
18	Paraproteins visible only by immunofixation should be described in the comment section (e.g., IgG kappa paraprotein only visible by immunofixation) rather than being given a quantified value
19	If a paraprotein is in the non-gamma regions, the beta region being the most common region for IgA paraproteins, report the total protein in the beta region (beta + paraprotein) quantification at presentation and during monitoring
20	The perpendicular drop method for quantification is proposed for gating of gamma-region paraproteins as opposed to tangent skimming or corrected perpendicular drop

21	The report should include a comment identifying the paraprotein as migrating in the beta-region and stating that the concentration includes normal beta proteins
22	Attempts to provide an estimate of the ‘true’ paraprotein concentration by subtracting a predetermined level for other beta proteins are inherently unreliable due to the non-constant levels of the co-migrating proteins and are not recommended
Urine paraprotein separation and quantification:	
23	First voided urine is suitable for screening UPEP
24	A 24-h urine specimen is preferred for staging and monitoring of the plasma cell dyscrasias, although first voided specimens are acceptable if a 24-h specimen is not available or practical
25	Laboratories should be able to detect BJP at a level of 10 mg/L with levels <10 mg/L reported as ‘trace’
26	While reporting the urine total protein, any intact monoclonal immunoglobulin should also be quantified and reported
Paraprotein characterization:	
27	IFE or immunosubtraction are required to characterize all new bands and to confirm their monoclonality
28	In subsequent specimens, IFE or immunosubtraction does not need to be repeated unless there is a change in the electrophoretic mobility, there is an additional visible band or if the paraprotein is no longer visible
29	Small paraproteins in the non-gamma region or in a polyclonal background also require IFE on each presentation in order to confirm their presence
30	IFE is required to confirm the absence of a previously reported paraprotein (to enable calculation of the response criteria ‘complete remission’). In general, once complete remission has been confirmed, IFE is not required on each subsequent occasion unless a new band is visible, or IFE is specifically requested
31	If the paraprotein is detected in the serum by immunofixation only, refer to this in the comment rather than in the quantification, e.g., ‘IgG kappa band visible only by immunofixation’
32	If the paraprotein is detected in the urine by immunofixation only, report this as ‘trace’ and refer to in the comment as only visible by immunofixation, e.g. ‘kappa BJP is only visible by immunofixation’
33	Preferably a final integrated report combining both the electrophoretogram and IFE should be issued
Laboratory performance of SPEP, UPEP and IFE:	
34	Preferably an assessment of laboratory performance of SPEP and UPEP requires determination of • analytical imprecision at different paraprotein concentrations to determine method repeatability and between-day and operator reproducibility. • limit of detection of protein electrophoresis and immunofixation. • the linear range of scanning densitometry.
35	A minimum competency-based standard is required for those who review and interpret protein electrophoresis patterns
36	Protein laboratories are encouraged to have an educational module suitable for continuing professional development
General Interpretive Commenting:	
37	**Normal pattern:** No significant abnormality is noted
38	**Decreased alpha-1 globulins:** Decreased alpha-1 globulins. Suggest alpha-1 antitrypsin quantitation if clinically indicated
39	**Decreased albumin and increased alpha-2 and beta globulins** is noted, advise to corroborate with serum lipid results to rule out nephrotic syndrome
40	**An increase in alpha-1 and alpha-2** fraction with a polyclonal increase in gamma globulin fraction is noted. Findings are suggestive of either chronic inflammation, chronic liver disease or autoimmune disease process.
41	**Increased beta-1 globulin (if IFE performed and paraprotein excluded):** Increased beta-1 globulin is noted, in absence of paraprotein on IFE, suggest to perform iron studies, if clinically indicated.
42	**Polyclonal hypergammaglobulinemia:** A polyclonal increase in gamma globulin fraction is noted. Findings are suggestive of either inflammatory process, liver disease or autoimmune disease process.

43	**Increased alpha-1 and alpha-2 and/or gammaglobulins:** Findings are suggestive of acute inflammatory process.
44	Beta–gamma bridging: **Hypoalbuminemia with a polyclonal increase in gamma globulin and beta fraction is noted. Beta gamma bridging is noted. Findings are suggestive of liver cirrhosis.**
45	**Hypogammaglobulinaemia (first presentation):** Hypogammaglobulinaemia is present. Suggest serum immunofixation and urine protein electrophoresis and immunofixation (or serum free light chains) together with quantitation of total serum immunoglobulins (if not already done/ordered)
46	**Hypogammaglobulinaemia (subsequent presentation):** Hypogammaglobulinemia is noted. Clinical correlation is indicated.
47	**Fibrinogen present:** Fibrinogen present. Please send repeat serum specimen. (No clinical comment is required if laboratory can run a repeat serum specimen, otherwise needs IFE to ensure small band is fibrinogen and there is no underlying paraprotein; optimally needs repeat serum specimen as a small paraprotein cannot be quantitated by agarose gel SPEP when masked by the presence of fibrinogen)
48	**Oligoclonal banding pattern with 2 or more bands on a polyclonal immunoglobulins background:** Oligoclonal bands are present. This can occur in a number of infectious or autoimmune conditions. Suggest review in 3–6 months if clinically indicated
49	**First detection of a paraprotein:** Suggest total serum immunoglobulins and urine protein electrophoresis and immunofixation (if not already done/ordered) [Typing and numerical quantitation, e.g. 'An IgG kappa paraprotein was detected in the gamma region']
50	**Follow-up of a known paraprotein which is still present:** Nil required. [A comment should be made on the original band and its current status, e.g. 'The previously reported IgG kappa paraprotein was detected']
51	**Paraprotein detected only by immunofixation electrophoresis:** The previously reported IgG kappa paraprotein is now only visible by immunofixation
52	**If paraprotein has disappeared:** A comment is required to confirm the absence of the previously detected paraprotein, e.g. 'The previously reported IgG kappa paraprotein was not detected by immunofixation'
53	**New, small abnormal band with different electrophoretic mobility from the original paraprotein in a patient with a known paraprotein:** There is a small (type: e.g., IgG kappa) band approximately (amount: e.g., 1 g/L) on a background of a polyclonal and/or oligoclonal pattern. This band is different from the original paraprotein. Its clinical significance is uncertain
54	**First presentation of small abnormal bands in polyclonal/oligoclonal background (and no known paraprotein):** A faint band is observed in the gamma region. In case of first-time occurrence (without any previous clinical history of monoclonal band), these may occur due to infectious and/or autoimmune diseases. These are often transient which may not require long-term follow-up, however serum immunofixation, urine protein electrophoresis & immunofixation is suggested to rule out any lymphoproliferative disorder. Follow up as monoclonal gammopathy of undetermined significance (MGUS) is suggested and repeat in 3-6 months' time period, if clinically advised.

Discussion

The adolopment of standardized reporting guidelines for SPEP and UPEP in Pakistan represents a significant milestone in enhancing the quality of clinical laboratory practices and ultimately improving patient care. This discussion aims to delve into the key aspects of our developed guidelines, their implications for the local healthcare landscape, and the potential benefits they offer pathologists, clinicians, and patients.

The absence of standardized guidelines for reporting protein electrophoresis in Pakistan has long been a concern, leading to variations in practices among clinical laboratories [15]. This issue has been particularly challenging given the limited resources and financial constraints prevalent in the healthcare system [16].

By synthesizing recommendations from a reputable source guideline and contextualizing them to the local healthcare dynamics, the developed guidelines address this critical gap and provide a framework for consistent and comprehensive reporting of PEP results. The process of guideline development involved meticulous review, expert consultation, and iterative refinement of recommendations to ensure relevance and applicability to the Pakistani healthcare setting [17]. The involvement of a team comprising the Consultant Chemical Pathologists and senior technologists underscores a collaborative and evidence-

based approach to guideline formulation. Senior technologists are experts with greater than 10 years of experience working at the bench with PEP, who were included in the team to provide a technical perspective when developing our recommendations. Furthermore, the adaptation and modification of recommendations based on expert consensus highlights the responsiveness of the guidelines to local healthcare infrastructure and resource constraints.

The modification made to recommendation 4 reflects a practical approach to address the resource constraints commonly encountered in low-income settings, such as Pakistan. In the initial recommendation, there was an emphasis on the necessity of a high-resolution electrophoretic system to ensure the detection of small monoclonal bands, especially in the beta region, which may co-migrate with normal proteins. However, acknowledging the reality of healthcare infrastructure in resource-limited settings, where high-resolution electrophoretic systems may not always be readily available or feasible to procure due to cost constraints, a revision was made to the recommendation to recognize the acceptability of low-resolution electrophoresis on cellulose acetate in situations where a high-resolution system is not accessible.

The initial recommendation 26 from 2012 suggested reporting the urine total protein and indicating the presence of glomerular and/or tubular proteinuria. Additionally, there was a directive to comment on the detection of Bence Jones Protein (BJP) and to quantify and report any intact monoclonal immunoglobulin found in the urine specimen. However, by focusing on reporting essential parameters according to the consensus of our local experts, the revised recommendation in the second version of the guidelines emphasizes a more streamlined approach. Hence, our modified recommendation maintains the importance of reporting urine total protein and quantifying and reporting any intact monoclonal immunoglobulin, while removing the specific indication for glomerular and/or tubular proteinuria and the comment on the detection of BJP. This revision ensures that laboratory reports remain informative and actionable for clinicians, even in contexts where comprehensive testing may be challenging to implement.

While the initial directive in recommendation 33 prioritized issuing integrated reports combining electrophoretogram and IFE for optimal patient management, the revised recommendation introduces flexibility by using "preferably." This acknowledges feasibility challenges in low-income settings and ensures diagnostic information is still provided despite constraints. It aligns with best practices while accommodating practical realities. The same rationale was implemented for recommendation 34.

The modification made to recommendation 37 reflects a shift towards a more concise and generalized interpretive commenting approach. In the initial recommendation, there was a specific mention of a "Normal pattern: Normal pattern. Paraprotein not detected," which provided a detailed interpretation of the electrophoretic pattern. However, recognizing the need for streamlined reporting practices that are both effective and efficient, the revised recommendation simplifies the interpretive comment to "Normal pattern: No significant abnormality is noted."

While the initial recommendation 39 provided detailed insights on nephrotic syndrome patterns and suggested corroborating with serum lipid results, the revised version simplifies language and removes explicit mention of syndrome consistency. By advising to corroborate with serum lipid results without specifying the pattern, the revision maintains clinical relevance while reducing the need for specialized interpretation, ensuring reports remain informative despite practical constraints.

Two recommendations were combined and refined to develop recommendation 54 in order to reduce redundancy and address potential diagnostic uncertainties. While the first recommendation addresses the presence of small abnormal bands in a polyclonal/oligoclonal background and suggests further testing to ascertain their clinical significance, the addition of the second recommendation acknowledges the possibility of faint bands observed in the gamma region without a known clinical history of monoclonal band. This addition provides additional guidance on the interpretation of such findings, suggesting considerations for infectious and/or autoimmune etiologies and emphasizing the importance of follow-up testing to rule out lymphoproliferative disorders. Hence, this guideline serves to offer a more comprehensive approach to interpreting electrophoretic patterns, thereby enhancing diagnostic accuracy, and facilitating appropriate clinical management.

10 recommendations were excluded from the final guidelines due to their infeasibility within the Pakistani healthcare system. For instance, the recommendation regarding the referral of problematic samples requiring the identification of small protein bands to a reference laboratory for isoelectric focusing (IEF) was deemed impractical due to logistical challenges and its limited commercial availability [1]. Additionally, the requirement for creatinine measurement on first voided urine specimens and the expression of BJP concentration relative to urine creatinine (BJP/creatinine) in mg/mmol was excluded as it may pose logistical and financial burdens on laboratories, especially in resource-limited settings where access to specialized equipment and reagents may be limited. These exclusions were necessary to ensure that the guidelines remained feasible and applicable within the context of the Pakistani healthcare system, while still providing valuable guidance for clinicians and laboratory professionals.

The finalized recommendations encompass a wide range of aspects related to pre-analytical, analytical, and post-analytical processes, including specimen collection and handling, instrumentation, interpretation of electrophoretic patterns, and reporting formats. Key revisions to the recommendations reflect a pragmatic approach to navigating resource constraints, such as the acceptance of low-resolution electrophoresis systems in case of unavailability of high-resolution systems. Our developed guidelines aim to ensure that laboratories in low-income

settings can still perform protein electrophoresis using available resources without compromising the integrity of diagnostic assessments.

Conclusion

The development of standardized reporting guidelines for SPEP and UPEP in Pakistan marks a significant advancement in laboratory practices, particularly within resource-limited settings. These guidelines, tailored to the local healthcare environment, ensure consistency and clinical relevance in protein electrophoresis reporting. Key revisions address practical constraints, such as the use of low-resolution systems and streamlined interpretive comments, ensuring laboratories can maintain diagnostic integrity even with limited resources. This initiative is poised to enhance diagnostic accuracy, support informed clinical decisions, and ultimately improve patient outcomes in Pakistan and similar contexts. Ongoing evaluation will be crucial to sustaining the guidelines' relevance and effectiveness.

Disclosures

None.

Funding

None.

Ethical Consideration

Not applicable.

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Brief Report

Integrating Patient-Generated Health Data from Mobile Devices into Electronic Health Records

Best Practice Recommendations by the IFCC Committee on Mobile Health and Bioengineering in Laboratory Medicine (C-MHBLM)

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Keywords

Wearable devices, Mobile health technology, Computer interfaces, Medical records

Abstract

Background

An increasing number of wearable medical devices are being used for personal monitoring and professional health care purposes. These mobile health devices collect a variety of biometric and health data but do not routinely connect to a patient's electronic health record (EHR) or electronic medical record (EMR) for access by a patient's health care team.

Methods

The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Committee on Mobile Health and Bioengineering in Laboratory Medicine (C-MHBLM) developed consensus recommendations for consideration when interfacing mobile health devices to an EHR/EMR.

Results

IFCC C-MHBLM recommendations cover personalized monitoring and privacy concerns, data security, quality assurance of data transfer, and incorporation of alert triggers to warn users of important health conditions.

Conclusions

Considerations for interface ease-of-use, display of patient data in the EHR/EMR, and needs-based training programs for healthcare staff to understand the critical requirements, proper

use, and integration of mobile health devices with EHR/EMRs are provided. Cooperation between healthcare providers, device manufacturers, and software developers is also recommended to drive future innovation in mobile health device technology development.

Introduction

The field of medicine stands as one of the disciplines most significantly influenced by the widespread availability of mobile devices. The use of mobile devices by health care professionals has transformed many aspects of clinical practice [1,2]. Mobile devices have become commonplace in health care settings and at home, leading to rapid growth in the development of medical software applications [3]. These tools can enhance patient experience, engagement, activation, and satisfaction by allowing patients to view and understand their health data through visual or auditory representations provided by the software applications [4,5]. Yet, we have not achieved a shared understanding of important mHealth constructs or how to conceptualize and operationalize them [5,6]. Patient centered mobile health (mHealth) is therefore seen as a challenging opportunity with still open questions related to the conceptual realization [5]. With all the new data available from these burgeoning mobile devices and their partner software applications, a challenge has arisen on how best to integrate these myriad data into a patient's electronic health record (EHR) or electronic medical record (EMR) to maximize positive clinical impact while minimizing complexity. Institutions may employ different EMRs that may not communicate with each other, while a patient's EHR may follow them between healthcare systems, state and international borders. These mobile health data recommendations apply to both EHRs and EMRs and are referenced as EHR/EMR in this guidance.

Healthcare data monitoring systems can be classified as follows: Remote Health Monitoring Systems (RHMS), which include systems that can send and/or receive their data remotely; Mobile Health Monitoring Systems (MHMS), an RHMS extension that uses smartphones or other mobile devices for local data processing on demand; Wearable Health Monitoring Systems (WHMS), where mobility is further enriched through wearable devices/sensors; Smart Health Monitoring Systems (SHMS), where "smart" denotes the approach and associated devices. In these systems, MHMS can leverage the local processing capabilities of mobile devices to analyze collected data and determine whether critical conditions exist. In such cases, an immediate alert is generated and communicated to medical staff, whereas normally, data upload is not done in real-time to reduce power consumption [7].

The World Health Organization defines mHealth as "medical and public health practice supported by mobile devices." Mobile health technologies refer to a variety of wearable devices that include "wellness devices" that monitor biometric and health data – heart rate, sleep, exercise and pedometers, "personal emergency response systems" – medical alert systems, dementia-related monitoring cameras, motion and fall detection, and "remote patient monitoring" – telehealth and medication tracking (telemedicine: healing from a distance) [8].

For these recommendations, mobile health technologies are digital applications, wearable devices and monitoring equipment that collect continuous or periodic data. This data is transferred and stored in the manufacturer's servers and can be accessed through software applications on a phone, computer, or other connected equipment, like Chromebooks or iPads, to allow for analysis and trending of personal health data.

Mobile devices and applications offer numerous potential advantages for healthcare professionals demonstrating their efficacy in enhancing clinical decision-making and fostering improved patient outcomes, whereby the effectiveness of health interventions based on mobile phone or tablet applications varies largely between indications [9,10]. Mobile health is a new way of communication, and we have not achieved a shared understanding of important mHealth constructs, or how to conceptualize and operationalize them [5,6]. Alongside the potential benefits, it is imperative to establish robust quality and safety standards, as well as validation practices, for mobile medical applications. This ensures their appropriate utilization and seamless integration into medical practice, especially considering the advancing sophistication of these tools. Recent advances in wearable devices have attracted significant attention due to their ability to provide continuous physiological information for continuous health monitoring by detecting biological signals. To make sense of the collected biological data and improve the effectiveness of these biosensors, scientists have integrated machine learning (ML) into wearables to analyze large data using various ML algorithms. Also, new information and communication technologies using the Internet of Things (IoT) have contributed significantly to integrating various areas of the healthcare sector with mobile technology. Thus, the technology could become a powerful medical tool to support the healthcare sector at all levels of care [11,12].

Wearable devices can provide real-time feedback regarding a person's health conditions; hence, they can provide an objective alternative to manage and monitor chronic disease progression, such as with the elderly, with rehabilitation, and for those with various disabilities. Wearable sensors are widely used in healthcare, due to their hardware capacity, small footprint and lower cost compared to equivalent medical instruments capable of monitoring the same vital signs. Furthermore. wearable technology decreases the cost of intensive treatment by allowing rehabilitation outside of the hospital in an ambulatory environment. According to recent estimates, wearable technology will flourish over the next 25 years, resulting in a global cost savings of over $200 billion in the healthcare industry and a considerable reduction in clinician/patient interaction time. Reports suggest that the number of wearable devices in use in 2020 was approximately 600 million, and current trends predict the number to increase to 928 million in 2021, and to reach 1100 million in 2022 [13].

What data is collected by mobile health devices?

The capability to download medical applications on mobile devices has unlocked a wealth of clinical and medical resources. These applications cover a wide range of functionalities, including electronic prescribing, diagnosis and treatment support, clinical guidelines, decision support aids, textbooks, and literature search portals. Mobile health devices collect biometric and personal health data from the wearer, such as heart rate, body temperature, activity (sleep/wake/exercise), alerts such as falls, and medication tracking. Some devices may collect analytes like glucose (CGM – continuous glucose monitors) through minimally invasive sensors that sample interstitial fluid under the skin or oxygen saturation through spectrophotometric scans of capillary blood under a wearable device. While called "continuous", mobile health devices sample the wearer, periodically or intermittently, every several minutes. Software applications can analyze data to calculate average, minimum/maximum, and trends, such as rate of rise or fall. The software can also predict future events, like hypoglycaemia, based on the rate of glucose fall and alarm the wearer before an event. Having alert system triggers for certain extreme or life-threatening conditions such as severe hypoglycemia, a patient fall, or significant cardiac arrhythmias, can activate a medical emergency response or follow-up for the affected patient. This may require having a command center that monitors those life threatening indicators around the clock and activate appropriate response when required. Biometric data can also be linked to information provided by the wearer about their health status through software applications - type of exercise, duration of exercise, sleep and wake times, meals and caloric intake, as well as menstruation cycle/fertility or general wellness (sick, fever, healthy).

Why is interfacing of mobile health data important?

Interfacing mobile health data is vital for advancing healthcare delivery, improving patient outcomes, and fostering innovation in medical research. It bridges the gap between technology and healthcare, creating a more integrated, efficient, and patient-centered healthcare ecosystem.

Several issues challenge the future integration of mobile devices and applications into health care practice. Mobile health devices don't currently interface with an EHR/EMR). While personal data is viewable by the device owner, software applications store the data in the manufacturer's computer servers rather than transmit the data to an EHR/EMR. This allows the manufacturer access to a tremendous amount (big data) of personal health information that could be mined for predictive health and population health trends. Yet, a person's primary health team can access only the data if trends are viewed from a software application during an office visit or data is printed as a summary report. Some institutions have developed research interfaces to devices, like CGM, that allow upload of a person's data during a healthcare visit. Still, routine interfacing of CGM and other wearable devices and monitors is not expected for a few years. Lacking an interface, many institutions scan printed summary reports into the EHR/EMR to allow clinicians access to the personal data trends. This is a manual process and can lead to lost data (if not scanned) or worse, the possibility of scanning data to another person's EHR/EMR. The security of personal health data is a concern for any interface that could allow computer hackers access to personal health data. So, encryption and other security measures to protect the confidentiality of health data must be considered. Fidelity of the data is also a concern during transmission to ensure that health data will be accurately recorded in the EHR/EMR. It should be pointed out that most of today's medical data lack interoperability: hidden in isolated databases, incompatible systems and proprietary software, the data are difficult to exchange, analyze and interpret. This slows down medical progress, as technologies that rely on these data – artificial intelligence, big data or mobile applications – cannot be used to their full potential [14].

Securing mobile devices is a complex task that requires constant vigilance. Although security technologies are advancing and healthcare professionals are increasingly focusing on cybersecurity, healthcare organizations must always prioritize data protection in an environment of growing threats. Basically, healthcare professionals are responsible for protecting the privacy, security, and confidentiality of electronic health information [15]. To counter this threat, it is essential to adopt effective mobile security solutions and implement new security measures as soon as they become available. Healthcare professionals and IT companies must also conduct regular audits to ensure the security of their systems and data.

Where should mobile health data reside and be displayed in the electronic medical record?

Biometric data collected from a personal mobile device should be recorded and displayed in the EHR/EMR where other vital signs such as pulse, heart rate, and blood pressure are recorded during a patient visit. It is essential to distinguish vital signs recorded by healthcare professionals from data received from a personal mobile health device. This is particularly important when mobile health devices collect analytes like glucose. The quality of clinical laboratories is highly regulated by the Clinical Laboratory Improvement Amendments law in the US and ISO standards and local regulations in various countries globally. So, the display of CGM data should be separated from the display of laboratory, blood gas, or glucose meter results since CGM is not regulated like a laboratory test. One possibility would be to display CGM data with other monitoring data, such as oxygen saturation from pulse oximeters. This would allow the separation of health data collected from personal mobile devices from regulated laboratory test results in the EHR/EMR. However, clinicians may want to monitor data side-by-side with laboratory results to compare trends - such as CGM trends displayed next to laboratory glucose trends. So, EHR/EMR support staff should develop future report displays that allow clinicians to customize their views of data while clearly labeling what information in

the EHR/EMR came from mobile health technologies versus regulated laboratory test results for future laboratory and healthcare inspections.

Key Recommendations

1. **Personalized monitoring and Privacy concerns**
Mobile health technologies empower people to take charge of their healthcare by monitoring personalized data about themselves. It also ensures compliance with privacy regulations and guidelines to safeguard patient confidentiality. EHR/EMR should comply to local laws or code of ethics for patient confidentiality, like HIPAA, "Health Insurance Portability & Accountability Act", an American federal law that sets standards to protect medical records and other personal health information.
2. **User Interface**
Biometric, monitoring, and other personal health data collected from mobile devices should be made accessible to the primary care team and clinicians respecting national legal regulations. User-friendly interfaces between mobile health devices and EHR/EMR should be developed.

3. **Data Security**
Implement robust data security measures to protect patient information during transmission and storage. International guidelines and cooperation should address encryption protocols, authentication mechanisms, and access control policies. Mobile health technology interfaces must ensure secure transmission and accuracy of data recorded in the EHR/EMR.

4. **Staff Training**
Develop targeted training programs for healthcare staff to effectively utilize and integrate mobile health devices with EHR/EMRs though not limited to device setup, data entry, troubleshooting, and data interpretation. Thus, training should emphasize the importance of distinguishing mobile health device data from regulated laboratory test results. Ensure mobile health data, alongside other monitoring data, like pulse oximetry and vital signs, is recorded appropriately in the EHR/EMR, and not in the laboratory result section of the EHR/EMR.
5. **Quality Assurance**
To establish quality assurance protocols to ensure the accuracy and reliability of data obtained from mobile health devices. This may involve periodic calibration, validation studies, and performance monitoring as per international standard or equivalent guidelines. Emphasize user education of self-calibrating mobile devices for regular correlation against laboratory testing or standard methods to be informed about the accepted tolerance for calculated inaccuracy in order to be empowered to make decisions to either correct for bias or replace the device.
6. **Alert Triggers**
Incorporate alert triggers for mobile health devices that target industry stakeholders, users, and regulatory boards:
   - Industry stakeholders must ensure consistency in data formats and metrics across various devices and develop seamless integration protocols for EHR/EMR systems to effectively incorporate mobile health data. Alert designs should be easily understandable and actionable for both healthcare providers and patients. Additionally, a robust framework for privacy and security to protect patient data is essential.
   - For users and patients, mobile devices must be user-friendly, with clear instructions accessible to individuals with varying levels of technical proficiency. It is crucial to provide resources and training that empower users to understand and respond appropriately to alert triggers. Alerts should deliver meaningful and actionable information, and there is a need for customer support to assist users in managing and interpreting these alerts.
   - By addressing key inputs and implementing recommendations, regulatory boards can work to develop minimum standards to enhance the effectiveness and reliability of mobile health device alerts and alert documentation, ensuring they provide valuable contributions to patient care and safety.

7. **Technology Development and Mutual Collaboration with Industry**
Encourage collaboration between healthcare providers, device manufacturers, and software developers to promote adherence to integration guidelines and drive innovation in mobile health technology for mutual progress and patient care benefit. Additionally, establish mobile health device standards based on best practice for industry stakeholders, users, and regulatory bodies to proactively address emerging issues, facilitate timely updates, and ensure compliance with evolving standards. This proactive approach ensures timely intervention, enhances integration processes, and supports continuous improvement in patient care and technology standards.

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Case Report

Retrospectively diagnosed familial hypocalciuric hypercalcaemia following total parathyroidectomy in an asymptomatic patient

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Keywords
asymptomatic hypercalcaemia, familial hypocalciuric hypercalcaemia, primary hyperparathyroidism

Abstract

Background
Familial hypocalciuric hypercalcemia (FHH) is a rare, benign condition that shares characteristics with primary hyperparathyroidism (PHPT), a more sinister condition that requires surgical intervention. This case report demonstrates misdiagnosis of FHH and highlights important learning points to prevent this in the future.

Case Presentation
Hypercalcaemia was incidentally discovered in a 21-year-old patient who had no symptoms of hypercalcaemia and no significant family history. Clinical examination was normal. Biochemical investigations revealed hypercalcaemia of 2.84mmol/L (2.15 – 2.50mmol/L) and hypophosphataemia of 0.71mmol/L (0.78 – 1.42mmol/L). Parathyroid hormone (PTH) concentration was mildly and inappropriately elevated (10.3pmol/L [2.0 – 8.5pmol/L]) triggering a suspicion of PTH-mediated hypercalcaemia. Parathyroid scintigraphy reported an ill-defined area of focal uptake above the left thyroid lobe. Fractional excretion of calcium estimations on 24hour urine collections were borderline (0.01) for FHH on multiple occasions however, further investigations to exclude FHH were not performed before a diagnosis of primary hyperparathyroidism was made, and a total parathyroidectomy performed. Several months post-operatively, the patient still demonstrated persistent hypercalcaemia. Her siblings had since been diagnosed with FHH. The patient was then retrospectively diagnosed with FHH. Genetic testing for FHH is not available in South Africa which limited the opportunity to confirm the diagnosis.

Conclusions
This case report provides a classical presentation of the rare, benign disorder of FHH. It highlights the negative outcomes that may result from misdiagnosis of this condition as PHPT. Biochemical investigations play an integral role in differentiating these conditions. Effective clinician-laboratory communication is crucial for optimal patient outcomes.

Introduction

Familial hypocalciuric hypercalcaemia (FHH) is a rare autosomal dominant condition caused by inactivating mutations in the calcium-sensing receptor (CaSR) gene.

FHH is characterised by lifelong hypercalcaemia which confers minimal, if any, morbidity [1]. Parathyroid hormone (PTH) concentrations may be normal or mildly elevated. Primary hyperparathyroidism (PHPT) is a relatively common endocrine disorder characterised by excess production of PTH and is associated with significant renal and skeletal complications over time [2]. While FHH shares features with PHPT, it is distinguished by demonstrating relative hypocalciuria for the degree of hypercalcaemia present. Family history, early age of onset and the lack of symptoms noted in FHH assists in differentiating it from PHPT, a distinction that significantly impacts appropriate management of the patient given that PHPT is treated surgically while FHH does not require treatment [3].

Case presentation

The patient is a 21-year-old female with no known co-morbid illnesses. She was incidentally found to be hypercalcaemic during baseline investigations performed for an elective procedure. No polyuria, constipation, abdominal or bone pain or any other symptoms of hypercalcaemia were reported. She had no previous history of renal calculi and no significant family history was noted. Clinical examination was normal.

Table 1: Blood investigation results.

Analyte	Result	Reference Interval
Urea	4.5mmol/L	2.1 – 7.1
Creatinine	88umol/L	49 – 90
Calcium	2.84mmol/L	2.15 – 2.50
Magnesium	0.98mmol/L	0.63 – 1.05
Phosphate	0.71mmol/L	0.78 – 1.42
Albumin	50g/L	35 – 52
PTH	10.3pmol/L	2.0 – 8.5
25-OH Vitamin D	43.80nmol/L	< 50.00 - Deficient

Laboratory investigations

Biochemical investigations revealed a hypercalcaemia of 2.84mmol/L (2.15 – 2.50mmol/L) and a hypophosphataemia of 0.71mmol/L (0.78 – 1.42mmol/L). PTH concentrations were mildly and inappropriately elevated at 10.3pmol/L (2.0 – 8.5pmol/L) leading clinicians to suspect a PTH-mediated hypercalcaemia. Vitamin D toxicity was ruled out with 25-OH Vitamin D concentrations which rather demonstrated a deficiency. Renal function was intact. Due to the young age of the patient and lack of symptoms, FHH was appropriately investigated for with the measurement of a urinary calcium: urinary creatinine clearance ratio (fractional excretion of calcium) on 24hour urine collections. This test was performed twice and on both occasions the clearance was borderline at 0.01. A fractional excretion of calcium <0.01 is indicative of FHH and >0.02 is suggestive of PHPT [4]. Parathyroid scintigraphy reported an ill-defined area of focal uptake above the left thyroid lobe. Skeletal survey and renal ultrasound confirmed the absence of skeletal abnormalities and renal calculi.

Table 2: Urine investigation results.

Test	Result	Reference Interval / Clinical Decision Limit
Urine Creatinine	2.7mmol/L	
Urine Calcium	0.99mmol/L	
Urine calcium: creatinine	0.37mmol/mmol creat	0.02 – 0.93
24-hour Urinary Calcium Excretion	0.01	< 0.01 – FHH > 0.02 - PHPT

Differential Diagnosis

A diagnosis of PHPT was made likely secondary to parathyroid hyperplasia or a parathyroid adenoma. This was based on the elevated PTH concentration and scintigraphy results.While molecular testing for CaSR gene mutations is not available in South Africa, FHH had not been excluded in this patient given the borderline calcium clearance ratio and clinical presentation. Testing of direct family members (parents and siblings) could have occurred to screen for asymptomatic hypercalcaemia with hypocalciuria. The patient underwent a total parathyroidectomy and all four glands demonstrated mild hyperplasia but no evidence of adenoma. Mild parathyroid hyperplasia is a feature in keeping with FHH [5]. The patient developed a mild hypocalcaemia post operatively but hungry bone syndrome, which is a relatively common post-operative complication of PHPT [4], was not observed. Several months post-operatively the patient was noted to have persistent hypercalcaemia which is a strong indicator of FHH rather than PHPT [6]. By this stage, the patient's siblings had been screened for FHH and three of the five siblings were found to have asymptomatic hypercalcaemia and 24-hour urine calcium clearance ratios <0.01 which confirmed the diagnosis of FHH in these siblings. A retrospective diagnosis of FHH was made in this patient. The patient and her siblings with FHH are reviewed annually and have been hypercalcaemic but asymptomatic to date.

Discussion

This case report highlights the role of the clinical laboratory in differentiating between two distinct conditions that share common features but are managed very differently – FHH and PHPT. It also demonstrates how lack of inadequate understanding of the pathophysiology of disease and its affect on biochemical findings may lead to misdiagnosis resulting in poorer patient outcomes. The raised PTH in the context of hypercalcaemia lead to a diagnosis of PHPT. However, consideration of the holistic picture, including pertinent history such as the age of the patient and lack of symptoms, and the borderline fractional excretion of calcium would have demonstrated the importance of excluding FHH in this patient, preventing an unnecessary surgical procedure. Consultation with the chemistry laboratory regarding further investigations to reach a definitive diagnosis may have been invaluable in this case.

Familial hypocalciuric hypercalcaemia (FHH) is a rare condition inherited in an autosomal dominant pattern equally distributed between the sexes. It's true prevalence is not known due to its subclinical nature in many cases [1]. It occurs as a result of mutations in the calcium-sensing receptor gene (CaSR) that lead to decreased receptor activity. The loss of function mutations in the CaSR gene in the parathyroid gland increases the set point for calcium sensing. It makes the parathyroid glands less sensitive to calcium, and a higher than normal serum calcium level is required to reduce PTH release. In the kidney, this defect leads to an increase in tubular calcium and magnesium reabsorption resulting in hypercalcemia, hypercalcaemia, and frequently high normal levels of serum magnesium [7]. Patients with FHH display higher levels of plasma PTH and it takes a higher level of plasma calcium to suppress PTH secretion.

Individuals with FHH will demonstrate lifelong hypercalcaemia, typically below 3.0mmol/l [5], as is seen in this patient with an inappropriately low urinary calcium excretion. Serum phosphate levels are often reduced, intact PTH levels are typically inappropriately normal in 80% of patients and mildly elevated in the remainder, and mild hypermagnesaemia may be present [6]. PHPT is characterised by excess PTH production by one or more of the parathyroid glands which can lead to significant skeletal, renal, abdominal, and neurological symptoms related to the resultant hypercalcaemia. PTH levels are often frankly elevated but may be within normal ranges in some cases [2].

Fractional excretion of calcium calculated from calcium and creatinine measurements on a 24-hour urine specimen and accompanying serum specimen can aid in differentiating the two conditions. Fractional excretion of calcium is calculated as [(24-hr urine calcium)/ (serum calcium)]/[(24-hr urine creatinine/ serum creatinine)], and a cut-point of <0.01 has been found to be indicative on FHH, while results >0.02 are more associated with PHPT [4]. However, 20-35% of patients with FHH may have a ratio above 0.01, and so genetic testing is recommended for those patients who fall within the "grey area" of 0.01 and 0.02 [6].

The patient had several features typical of FHH including young age, asymptomatic presentation, mildly elevated serum calcium (2.58mmol/l), low serum phosphate (0.71mmol/l), and mildly elevated PTH concentration (10.3pmol/l). Unfortunately, urinary fractional excretion of calcium was borderline at 0.01 and without the availability of genetic testing for mutations of the CaSR gene, a diagnosis of primary hyperparathyroidism was made based on the mildly elevated PTH concentration and parathyroid scintigraphy findings. While lack of genetic testing availability was a limitation in this work-up, it is a commonly encountered predicament in low-middle income settings like South Africa. However, other methods can be employed to assist in making a diagnosis, especially when differentiation of these conditions will have significant implications for patient management. Knowledge regarding the autosomal dominant nature of FHH could have been applied and testing of the immediate family members of the patient may have been useful pre-operatively, especially given that there was no indication to expedite the surgery. While this may not yield definitive results, it can be helpful and was eventually how the diagnosis was made. This case demonstrated that the differentiation of PHPT and FHH may still be a diagnostic challenge in some circumstances. Importantly, the chemistry laboratory was not consulted during the course of this case, and this collaboration may have led to a different outcome. It is likely that more emphasis would have been placed on reaching a definitive diagnosis before invasive interventions were performed. Thorough review of the clinical and radiological findings as well as biochemical investigations

would likely have led to the suggestion of postponing the surgery until screening of family members for FHH can occur or arrangements could be made to outsource the genetic testing for FHH to an overseas facility.

While there are several published case reports of FHH, none that demonstrated the misdiagnosis of FHH as PHPT were identified. A case of FHH reported by Al-Ramdhan et al [9], demonstrates how the diagnosis of FHH was made both by testing family members of the index patient with basic serum and urine biochemical investigations as well as with genetic analysis.

The strength of this case report is that it demonstrates a classical presentation of a rare disease as well as how an inadequately investigated diagnostic dilemma and misdiagnosis led to an unnecessary surgical procedure. The limitation of this case report is that the chemistry laboratory was not actively involved in the decision making regarding diagnosis and management of this patient as well as the lack of availability of genetic testing. Regardless, relatively simple, easily accessible biochemistry investigations were integral to the generation of a differential as well as the eventual diagnosis in this patient and several of her family members.

Learning Points

- FHH is a benign condition that can be distinguished from PHPT clinically and biochemically, preventing unnecessary surgical procedures
- Screening of family members for FHH using routine biochemical investigations can aid diagnosis when genetic testing is unavailable
- Effective and collaborative communication between clinicians and the laboratory is crucial for optimal patient outcomes

Abbreviations

FHH – familial hypocalciuric hypercalcaemia
PHPT – primary hyperparathyroidism
PTH – parathyroid hormone
CaSR – calcium sensing receptor

Author Disclosures

The author wishes to declare that she has no personal or financial interests that may have influenced the writing of this article.

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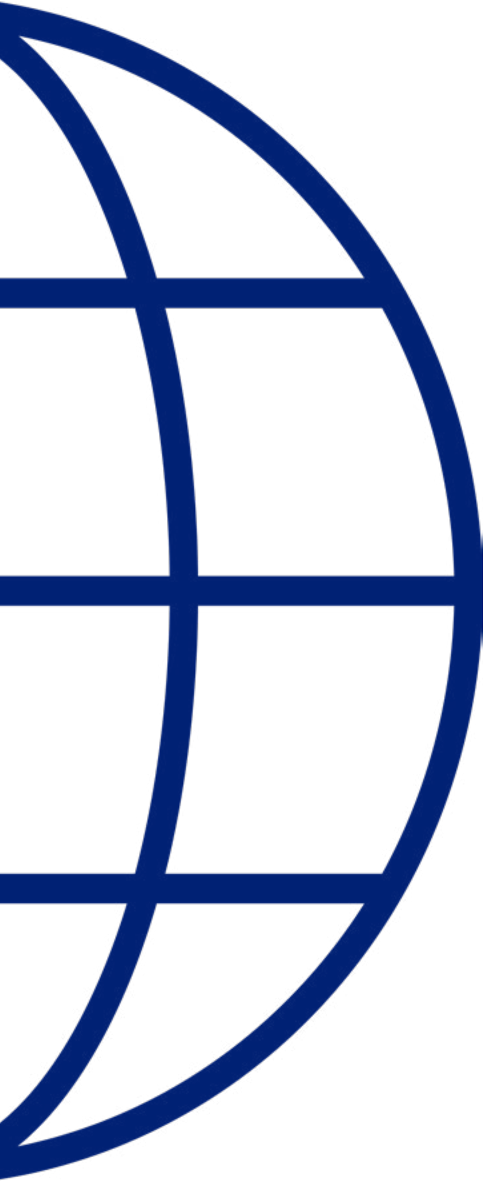

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