An-Najah National University

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IMPLEMENTING CYSTATIN C AS A BIOMARKER TO IMPROVE DIAGNOSTIC ACCURACY IN CHRONIC KIDNEY DYSFUNCTION IN PALESTINE

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Dedication

This thesis is dedicated to:

My brave and unwavering people, who face challenges with courage and determination.

my parents, (may Allah protect you and prolong your live),whose love and support have shaped my journey. Your unwavering support has been my greatest blessing.

my beloved sisters and brothers, whose love have been my foundation.

my husband, whose partnership and encouragement have illuminated my path.

my sons, who are my greatest motivation and joy, may you always pursue your dreams with passion and integrity.

To everyone who is dear to my heart, and to

every patient who suffers, i dedicate this work as an expression of solidarity and hope, and may your suffering be an inspiration for the power of change and healing. You are in our hearts and minds.

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Firstly, I must thank Allah for his graces and blessing on me to complete this work.

I would like to acknowledge my supervisor, Dr. Nihad Othman; thanks for your patience with me every step of the way.

I am grateful to everyone who helped me.

Declaration

I, the undersigned, declare that I submitted the thesis entitled:

IMPLEMENTING CYSTATIN C AS A BIOMARKER TO IMPROVE DIAGNOSTIC ACCURACY IN CHRONIC KIDNEY DYSFUNCTION IN PALESTINE

I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: **Malak Read Saleh**

Signature:

Date: **6/2/2025**

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IMPLEMENTING CYSTATIN C AS A BIOMARKER TO IMPROVE DIAGNOSTIC ACCURACY IN CHRONIC KIDNEY DYSFUNCTION IN PALESTINE

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Abstract

Background: Chronic kidney disease (CKD) continues to be a major threat to public wellness. Since the glomerular filtration rate (GFR) is a reliable indicator of kidney function and the traditional direct measurement techniques are expensive and time-consuming, estimation GFR has emerged as the most widely used technique for estimating renal function using endogenous chemicals like creatinine. Nevertheless, the restriction of creatinine requires the use of alternative markers for timely and accurate diagnosis.

Objectives: This study aimed to test cystatin C as a substitute biomarker for estimating GFR in CKD patients in Palestine and compare its efficacy to creatinine.

Methodology: This cross-sectional study was performed between June and December, 2023 focuses on CKD patients from clinics or admitted to the Ramallah Governmental Hospital and Al-Watani Government Hospital in Nablus, Palestine. A total of 160 patients were included. Demographic, medical data were collected using structured questionnaires and hospital records. Creatinine and cystatin C levels were performed from plasma samples of patients. Statistical analysis was performed using SPSS, employing Pearson's chi-square, with a p-value of less than 0.05, which was considered significant.

Results: The study's findings suggest that cystatin C may offer a more reliable estimation of GFR compared to creatinine, with a higher stability. The equation that used both serum creatinine and cystatin C was better than the equations that used only one of these markers, as it minimized the independent limitation effect of both markers alone.

Conclusion: Implementing cystatin C as a biomarker could enhance diagnostic accuracy for CKD, allowing for timely intervention and management, thereby improving patient outcomes and reducing healthcare costs associated with advanced renal failure. Further research is recommended to solidify these findings and promote the integration of cystatin C into routine clinical practice.

Keywords: Chronic kidney disease, Glomerular filtration rate, Cystatin C, Creatinine, Chronic kidney disease epidemiology (CKD EPI) equation.

Chapter One

Introduction and Theoretical Background

1.1 Overview

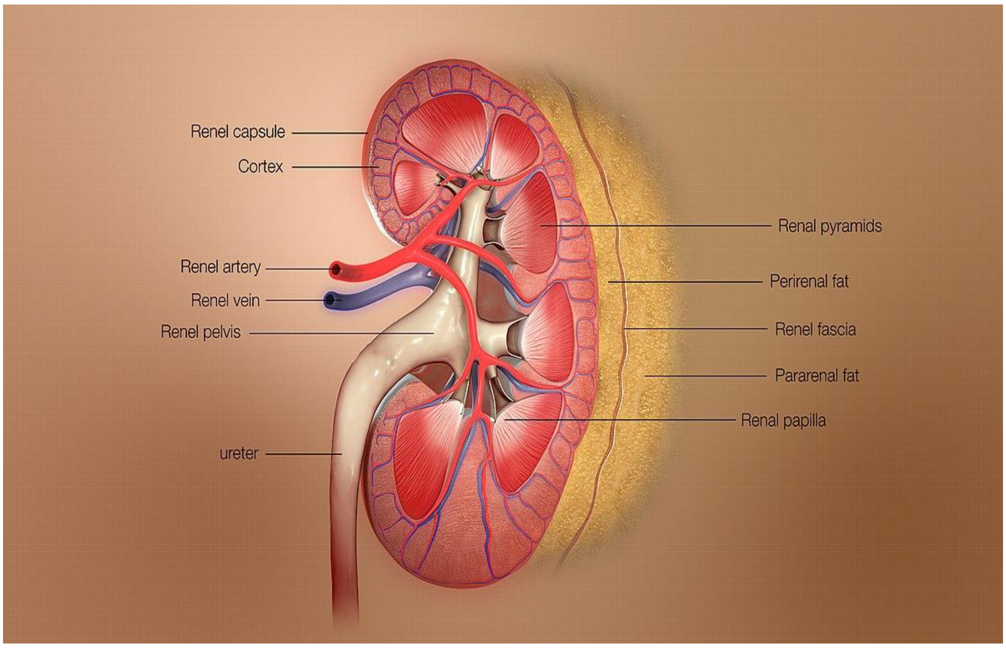
The kidney is a vital organ with sophisticated anatomy, approximately the size of a fist and resembling a reddish bean in shape [1]. Most humans have two kidneys serving as excretory organs. The right one is positioned lower than the left in the back of the belly, behind the peritoneum [2]. The four major anatomical regions that make up the kidney are the outer cortex, the medulla, the renal papilla, and the renal pelvis, all covered by a soft fibrous capsule coated in a layer of fat. The renal fascia borders the perinephric fat to keep the kidneys healthy from trauma [1, 2].

The functional elements of the kidney, found in the cortex and medulla, are called nephrons [3]. Nephrons consist of a renal corpuscle, a glomerulus that acts as a filtering unit encased in a Bowman capsule, and renal tubules, essential for returning vital nutrients [4]. The renal artery arises from the abdominal aorta and has a double capillary pattern. The afferent arterioles, which form and feed the glomerular capillary and can deal with high hydrostatic pressures, are the main force for filtration. Then blood flows from the glomerular capillaries directly to the efferent arterioles, which further divide to form the peritubular capillaries that have low hydrostatic pressure, making them able to remit the reabsorbed fluid and electrolytes to the circulation [1, 5]. This contrast in blood pressure caused by the afferent arterioles and efferent arterioles determines the kidney's filtration capacity [6].

The glomerulus is the first part of the nephron that filters plasma to produce urine. It is formed from a network of capillaries slightly coated in fenestrated endothelial cells, forming the inner glomerular filtration barrier. This layer is characterized by its high water permeability and ability to allow the passage of protein with low molecular weight and blood components other than cells. A glomerular basement membrane separates this inner layer from the outer layer, a negatively charged physical noncellular barrier that prevents proteins from penetrating the glomerular filtration barrier [1, 4, 7]. The glomerular capillaries' third and outer layer consists of visceral epithelial cells called podocytes. They represent a significant portion of the ultrafiltration system due to cellular structure that includes a cell body and processes that project outward forming foot processes that encircle the glomerular capillaries and create a more selective filtration barrier [8]. The glomerulus filters the blood passively through hydrostatic pressure. Without energy, the amount of fluid and solute crossing the filtration membrane is determined by the capillaries' outward and inward force [7]. The proximal tubules reabsorb over 70 percent of the glomerular filter fluids and solute, accounting for the high energy requirement in this nephron segment, whilst the distant nephron segments are crucial for urine concentration and salt excretion control [9].

Figure 1

Anatomy of kidney



Even though the kidneys are small, with an average of 0.65 percent of the body weight, they play a significant role in the body; a quarter of the cardiac output goes to the kidneys [2, 10]. The cortex receives eighty-five percent of the blood flow, the outer medulla receives fourteen percent, and the inner medulla receives one percent [1]. By filtering fluids daily from the renal blood flow, the kidneys maintain vital nutrients in the blood while allowing poisons, metabolic byproducts, and extra ions to be expelled [7]. The kidney controls the plasma osmolar by adjusting the blood's water concentration, solutes, and electrolytes. It also generates renin to control blood pressure, transforms inactive vitamin D into a functional form, and produces growth factors, prostaglandins, and arachidonate metabolites. Additionally, it maintains acid-base equilibrium and increases red blood cell creation via erythropoietin generation [7, 10].

1.2 Pathophysiology

The physiological functioning of the kidneys is linked to crucial metabolic processes; therefore, if the kidneys are subjected to adverse conditions, such as severe and extended oxidative stress, their normal function may be lost, which disrupts the body's equilibrium [11]. As a result of its exposure to medications, xenobiotics, and toxins, due to the high blood flow that reaches the kidneys, as well as their high oxygen consumption, the kidney is frequently a target organ of toxicity and harm [1]. Additionally, the pro-inflammatory response to infection and consequent kidney tissue destruction, whereas infectious microorganisms have been linked to kidney illnesses by directly causing kidney damage or generating immune-mediated damage [12]. Also, sepsis-related failure of multiple organs might involve kidney damage. In contrast, the renal system collaborates with almost every organ system in the body by maintaining fluid balance and normal function of organ systems. Damage to the kidney can cause injury to organs, especially the heart, lungs, digestive, and hepatic systems, through a variety of pathways. On the other hand, kidney impairment can be caused by malfunctioning any organ system [13].

The clinical manifestations may be acute or chronic depending on the status, symptoms, and underlying cause of the kidney disease. Acute kidney injury is marked by a rapid reduction in renal function, which causes waste products to be retained, and according to clinical studies, acute kidney disease increases the risk of developing chronic kidney disease [14]. Otherwise, CKD is marked as a gradual deterioration of kidney function that results in renal replacement therapy, either dialysis or transplantation, due to irreversible nephron loss [15]. The consequences of chronic kidney disease go beyond progression to kidney failure and elevated risk of cardiovascular disease but also reduce the efficiency of kidney function as well as anemia owing to decreased erythropoietin synthesis by the kidney, diminished red blood cell survival and iron insufficiency, bone disease due to mineral disrupted, secondary hyperparathyroidism, fluctuations in the electrolyte and water balance, metabolic acidosis, poor quality life also has a significant impact on mortality around the world [16, 17].

1.3 Etiology and Prevalence

The incidence of CKD patients is further increasing, affecting an estimated 843.6 million individuals globally in 2017 due to the increase of the risk factor [18]. According to the impact of disease research globally, there has been a 32% increase since 2005, with studies showing that 1.2 million people died from kidney failure in 2015 [16]. In the Arab world, relatively little information is available on the precise prevalence of different kidney illnesses [19]. The prevalence of end stage renal disease among Palestinians who are on dialysis was 240.3 per million population in 2010 [20]. According to the Palestinian Central Bureau of Statistics (PCBS), the proportion of people who are 18 years of age or older and have at least one chronic illness has climbed somewhat over the previous ten years, reaching 18% in 2010 and almost 20% in 2021.

Outside of normal aging, renal system defects can affect anyone at any time and age [21]. It's noted that the prevalence of chronic kidney disease around the world has been increasing rapidly recently. It may be due to the lifestyle adopted and genetic factors [22]. Chronic kidney disease is a severe public health issue. It is defined as the presence of kidney damage or decreased kidney function due to structural, functional abnormalities, or both [23]. One of the critical causes of chronic kidney disease, along with hypertension, is hyperglycemia; additional important risk factors include obesity, unconditional uses of pain relievers, and a sedentary life style, all of which are common in Arab nations [19]. In 2015, the prevalence of type 2 diabetes mellitus among Palestinians in the West Bank was 18.4%. These percentages are expected to rise to 23.4% by 2030 [24]. It was also found that approximately 24% of those with diabetes suffer from complications of chronic kidney disease [16]. Although there are insufficient statistics in Palestine on chronic kidney disease, the rate is expected to increase significantly in recent times due to the restrictions imposed on the health infrastructure that the Palestinian people suffer from as a result of the ongoing Israeli war.

While the etiology and development of CKD are complex and not fully understood, several factors such as age, smoking, and BMI, as well as the coexistence of chronic conditions and the longevity of illness term, are positively connected to CKD [23]. In addition to the psychological effects, stress, anxiety, and sadness resulting from the consequences and scenes of war, which leads to high blood pressure, which is a significant factor in the development of kidney disease.

1.4 Chronic Kidney Disease Pathogenesis

There are a variety of renal disorders, starting with biological and molecular alterations that progress into cellular damage [14]. A number of underlying reasons can lead to the onset of chronic kidney disease, which could be an abnormality during development, a congenital disability, a hereditary cause, or the outcome of a lifelong injury, whether from immune-mediated, inflammatory, or toxicity causes [25]. In response to damage, the kidney tries to renew and repair nephrons; however, the repair process may not be appropriately guided, affecting the nephron function [26]. The remaining ones frequently induce hyperfiltration and hypertrophy to adapt. While this adaptation aids in the maintenance of kidney function in the short term, it may be detrimental in the long run as the increased workload on the remaining nephron can result in ongoing dysfunction and a progressive reduction in total renal function, potentially causing more severe consequences [27]. By adjusting the diameter of the afferent and efferent arteries, the kidney could decouple glomerular filtration from fluctuations in blood pressure and react quickly to environmental changes [28]. Similar to the usual number of nephrons in people who are in good health, vasodilation occurs in situations of increased burden, such as large protein consumption, obese, diabetic patients, and pregnancy, leading to a rise in GFR in the single nephron and absolute hyperfiltration [29]. In the same direction, GFR is influenced by the actual filtration pressure, the total amount of surface area that may be used for filtration, and the permeability of the filter membrane [5]. In pathological conditions in patients with a reduced number of nephrons, either from birth or with an acquired reduction as a result of kidney disease, a relative hyperfiltration occurs by a decrease in the arteriole diameter, resulting in an increase in single nephron GFR as an attempt to preserve renal function [30]. Vasodilation of the afferent arterioles causes a decrease in resistance and an increase in GFR as a result of elevated hydrostatic pressure, while efferent arteriole vasodilation also causes a reduction in resistance but, in contrast, decreases the capillary pressure and the GFR; the flip side happens in vasoconstriction [28]. The kidney's compensatory increase in GFR per nephron in the remaining functional nephrons may render the initial consequence of early nephron loss undetectable [31].

1.5 CKD Diagnosis

Chronic kidney disease is shown by a decrease in glomerular filtration rate persisting for three months or more, with eGFR less than 60 ml/min/1.73 m2. It is classified by its severity into five stages, as shown in table (1), based on the filtration rate, whereas a GFR of 120 to 125 ml/min serves as usual [23]. It is the best predictor of renal function. It is defined as a measurement of how much plasma is filtered through the glomeruli per minute in all functional nephrons. The quantity of plasma totally cleared of a substance in a certain amount of time is referred to as "clearance" [32, 33]. A substance readily filtered in the glomerulus, is not released or reabsorbed by the renal tubule, is not metabolically altered along its transit, and is not albumin-bound satisfies the prerequisites for an ideal filtration marker [34]. Although there are several protocols available for evaluating kidney function tests, and they differ in terms of practicality, accuracy, and sensitivity, it is necessary to use biomarkers that offer quick, non-invasive, precise assessments that are well correlated with kidney tissue pathology to monitor renal function in chronic kidney disease and also fulfill the ideal marker criteria [32].

The majority of persons at the stage of decreased glomerular filtration rate die of cardiovascular disease before they experience renal failure. This is because CKD typically has no symptoms, develops slowly, and is frequently misdiagnosed or discovered too late. On the other hand, CKD can progress to end-stage renal disease in a small but significant percentage of people, as they are five to ten times more likely to die before developing end stage renal disease [35, 36]. Also, people with renal failure are rising, and those patients must usually be referred for dialysis or transplantation as a kind of renal replacement therapy, and the expense of this therapy is often out of reach for many people. In addition, the evolution of CKD to end-stage leads to poor clinical outcomes with enormous human and financial consequences [37]. Many CKD consequences can be avoided or delayed if diagnosed early. A growing body of research suggests that a considerable percentage of individuals with early-stages of CKD are underdiagnosed. Early identification of renal illness can help postpone or prevent kidney function degradation with low-cost therapies [38].

Table 1

Categories of Chronic Kidney Disease Based on GFR

GFR category	GFR (ml/min/1.73m2)	Term in kidney function
G1	≥90	Normal or High
G2	60-89	Mildly decreased
G3A	45-59	Mildly to moderate decrease
G3B	30-44	Moderate to severe decrease
G4	15-29	Severely decrease
G5	<15	Kidney failure

GFR: glomerular filtration rate, G1: stage one, G2: stage two, G3: stage three, G4: stage four, G5: stage five

A range of tests performed in clinical laboratories help examine and assess kidney function. In clinical settings, estimating the glomerular filtration rate and looking for proteinuria are the most practical methods to evaluate renal function [39].

1.6 Problem Statement

The prevalence of ESRD among Palestinians is rising; early diagnosis and accurate staging are required due to the sharp rise in the prevalence of kidney dysfunction and the high cost of treatment. The GFR is the greatest indicator of kidney function. However, the difficulty and expense of direct measurement in clinical practice restrict the method used and increase the researcher's interest in finding the easiest and most accurate method to evaluate GFR. One of the limited methods is radiation exposure while measuring the glomerular filtration rate using isotopes. The radiocontrast agents containing iodine can cause allergic responses in some people, so multiple short-term exposures are not recommended.

At the same time, exogenous methods for measuring GFR are more precise, but they are also time-consuming, expensive, and challenging to use widely. Also, these techniques are not practical at renal clinics and are unavailable in Palestine. Therefore, estimating GFR by an endogenous biomarker is routine practice's most frequently used surrogate marker. Creatinine concentrations are commonly used clinically as an indicator of kidney health. It is well known that blood creatinine and urine creatinine clearance have limitations when used to estimate GFR. The factors unrelated to GFR, such as age, race, muscular mass, gender, medication, and catabolic condition, impact creatinine value. Meanwhile, the overall rate of creatinine synthesis depends on the body's mass, declines with age, and is lower in females than males.

Also, inaccurate data is still available for creatinine clearance due to collection issues and variations in creatinine excretion. Currently, the equations that estimate the glomerular filtration rate (GFR) that use creatinine and creatinine clearance are commonly used approaches for determining GFR. However, they still have a vulnerability gap since eGFR based on these equations does not consider the expected normal aging and sex-related decrease in GFR in great detail. Many older adults, especially those who are not at risk, may be classified as having CKD. Moreover, before serum creatinine significantly increased in the early stages of acute renal failure, eGFR-based creatinine can be dramatically overestimated from Scr. In another way, the main issue was probably the impact of creatinine technique bias on the estimated GFR. As the scope of CKD became apparent, concerns about its over-detection emerged.

Cystatin C, which can be detected in serum, has lately been recognized as a great substitute. The benefits of cystatin over creatinine include the fact that it is unaffected by age, muscle mass, or nutrition. It is a more accurate indicator of GFR than creatinine, particularly in the early stages of renal impairment. However, its adoption has been hampered by apparent rising prices and medical inexperience.

1.7 Study Hypothesis

- Alternative non-directional hypothesis:
  Cystatin C is a better predictor marker than creatinine in eGFR in the early stage of CKD.
- Null hypothesis:
  Cystatin C is not a better predictor marker than creatinine in eGFR in the early stage of CKD.

1.8 Objectives and Aims

1.8.1 Main objective

The main objective of this study is to test cystatin C as a new estimated biomarker for eGFR in chronic kidney disease patients in Palestine. The study also seeks to evaluate kidney function by using the CKD EPI-based cystatin C equation and comparing the outcomes with creatinine assays of the participating patients to determine if cystatin C as an alternative to creatinine has a greater capacity to predict the risk of adverse outcomes according to the current research..

1.8.2 Specific Objectives

1. Test the cystatin C as a new, sensitive biomarker for early detection of kidney disease in Palestine.
2. Evaluate the advantages and disadvantages of employing cystatin C as a kidney function biomarker. (Is cystatin C a sensitive biomarker in eGFR compared to its cost, the method, and the duration of measurement)?
3. Determine if cystatin C is a superior alternative to creatinine in terms of its ability to predict kidney problems in their early stages. This will be achieved by comparing the performance of cystatin C and creatinine, incorporating the results into eGFR estimation equations, and analyzing the outcomes.

1.9 Literature Review

1.9.1 Glomerular Filtration Rate Estimation

The glomerular filtration rate is the amount of circulatory fluid filtered by the renal cells per minute. It is the most reliable predictor for evaluating renal function, identifying, staging, and managing chronic kidney disease, and establishing doses of medication [39]. Urinary clearance was the primary and frequent way of determining glomerular filtration rate [40]. Clinically, this approach becomes impractical for regularly monitoring kidney function despite its simplicity and affordability due to the need for a 24-hour urine sample [34].

GFR can be calculated by tracking the clearance of substances from plasma, which may be endogenous or exogenous [41]. The most reliable method for determining GFR is to measure the plasma of an exogenous marker, such as inulin or iothalamate, because it fulfills the standards of an ideal filtration [31]. This method needs injecting the exogenous marker subcutaneously to get more consistent plasma by a gradual diffusion of the marker, then requires approximately one hour to reach equilibrium before collecting blood samples. The necessity of intravenous injection and a subsequent blood sampling at a specific time, this in addition to its high cost restricts it from being regularly used [32]. In the clinical context, the preferred alternative is to calculate GFR based on measuring the concentration of an endogenous biomarker in the serum, such as creatinine or cystatin [42].

1.10 Endogenous Glomerular Filtration Rate Indicator

1.10.1 Creatinine Test

Creatinine has been the most widely utilized eGFR endogenous biomarker over time, and it represents either renal clearance or serum concentration. It is produced at a constant rate under a normal state, freely filtered due to its small size, and not bound to any proteins. It is not reabsorbed. This, in addition to its measuring method, is simple, not complicated, and is not expensive [43]. Creatinine is a final product of creatine and creatine phosphate of muscle metabolism. Creatine is produced mainly in the kidneys and liver, then transported and stored in muscle cells, where creatinine is produced spontaneously and nonenzymatically based endogenous production.

Additionally, it is produced by the body naturally and is present in cooked meat; therefore, having a large meal of cooked meat could also increase and impact serum creatinine levels [44]. Although blood creatinine rises as a result of reduced renal clearance, it is also elevated by greater muscle mass. Since serum creatinine is a byproduct of muscle metabolism, its fluctuation depends not only in conjunction with GFR, which poses a significant restriction to this test in renal evaluating in individuals with exceptionally low or high muscular mass as in eating disorders, overweight, muscle wasting disease, and bodybuilding [45]. Creatinine is not the best filtration marker, where many variables GFR independent affect creatinine, such as age, gender, diet, physical activity, and tubular secretion, where a portion of creatinine, as much as fifteen percent, is secreted into the tubules from the peritubular capillaries so the kidneys eliminate more creatinine than glomerular filtration does on its own. This why creatinine clearance overestimates the absolute GFR, and thus rise in serum creatinine may be suppressed until GFR has nearly fifty percent [6, 46]. Also, some medications have an effect that prevents the tubular release of creatinine, lowering creatinine clearance and increasing the serum creatinine level without changing the GFR [43]. It should be taken into consideration the extrarenal clearance of serum creatinine, regardless it is negligible within normal circumstances, but when GFR decreases, creatinine intestinal excretion increases, in which intestinal flora degrade it to creatine, also its notable variations in creatinine generation in some disease as in liver dysfunction patients there is a decrease in creatinine production [44]. These explanations explain why there are significant individual and temporal variations in the association between serum creatinine levels and GFR. Despite relying on creatinine in GFR estimation until this time, it should be noted that whereby before creatinine levels rise, the glomeruli lose at least 50% of their function, also considered suboptimal in CKD screening for elderly patients and women due to the low of creatinine production and also patients with early stage of CKD go undetected [31]. Although methods for measuring creatinine are constantly improved analytically, they still have poor sensitivity, specificity, and interference with another analyte. It might be deceptive to use a single reference range for serum creatinine to differentiate between an abnormal and normal GFR [43, 47].

Meanwhile, creatinine clearance enhances the estimation of GFR and removes some issues with serum creatinine levels. It has a significant drawback; one of its main issues is the overestimate of the truth GFR due to tubular secretion, as well as its influence on the incorrect collection and preservation of urine samples; thus, the use of creatinine-based estimated GFR equations overcomes these drawbacks and has increased the validity of serum creatinine, which is regarded as an insensitive measure of glomerular function when used alone; also, the eGFR equation yields a more precise estimate of GFR; its inferred from the blood levels of endogenous filtration indicators without clearance calculating and its useful in daily uses [31, 42]. It is important to pay proper consideration to clinical and statistical methodologies when developing and validating GFR estimation equations. Enhancing eGFR precision may have an impact on the health of many people [42].

1.10.2 Creatinine Equations Based GFR Estimation

1.10.3 Cockcroft–Gault Equation

Cockcroft–Gault (C-G) and the Modification of Diet in Renal Disease (MDRD) study equations were the most common creatinine-based GFR estimation formulas that have been frequently used and widely studied [41]. Cockcroft–Gault was devised in 1973. It was among the first prediction formulae. It also helps in tracking changes in a patient's renal function, despite its tendency to overstate renal function, especially in patients who had ascites or severe obesity and also in cases of muscle wasting related to age and body weight, where serum creatinine is abnormally lower than expected resulting in unrealistically high eGFR-based creatinine [41, 46]. Additional negative aspects of this equation include that it was conducted before the standard procedures for creatinine evaluation were developed [43].

$$eGFR \ = [(140 - age) \times weight]/(72 \times Scr)[\times 0.85 \ if \ female] \qquad 1$$

Where age is expressed in years, weight in kilograms, and serum creatinine (Scr) in milligrams per deciliter [48]. The limited regular use of the Cockcroft and Gault equation in laboratory practice is due to the necessity of calculating body weight. In contrast, the simplicity of the MDRD equation, which does not require body weight to calculate eGFR, makes the probably of mistakes due to fluid overload and obesity less likely, which helped this formula's rapid introduction and acceptance into clinical practice [46, 49].

Compare between GFR measured by using the gold standard inulin marker approach and GFR estimated according to creatinine formulas, it found that the eGFR equation was significantly overstated inulin clearance, and comparing Cockcroft & Gault and MDRD formulae, a significantly overestimated GFR was noted. However, there was no statistically significant difference between their mean values. The MDRD equation showed a somewhat better but not statistically significant improvement in GFR estimation compared to Cockcroft & Gault [50]. Also, compared eGFR using creatinine equations, the C&G and MDRD formulas in stage 2 and stage 3, GFR 30-89 ml/min/1.73 m2, it is found that the MDRD formula was considerably more accurate than the C&G formula [51].

1.10.3.1 Modification of Diet in Renal Disease (MDRD) equations

MDRD was developed in 1999 as an essential test in determining renal function, but it is constrained by decreasing the GFR at higher levels. Its effectiveness is poorer in individuals with normal GFR due to its exclusive development in renal impairment cases, which may cause the prevalence of stage G1-G3 of CKD to be exaggerated [49]. On the other hand, its accuracy is higher in individuals with more damaged kidneys.

The MDRD estimating equation is

$$GFR = 186 \times (Scr \times 0.0113) - 1.154 \times (age) - 0.203 \times 0.742\ (if\ is\ female) or \times 1.212\ (if\ is\ black). \quad (2)$$

To improve the limitations of the MDRD equation, specifically the lack of accuracy and the underestimation of the eGFR at higher levels due to its origin from a sample of CKD patients, and its value for people in good health is unknown [52]. The CKD-EPI equation has been proposed to replace it in clinical use, as it was developed from the MDRD formula in 2009. It was measured in relation to iothalamate clearance and comprised both kidney disease-related and non-related participants [49]. It is also applicable to all phases of kidney failure, and considering fewer biases, improved accuracy, and greater precision, the revised formula outperformed the MDRD study formula significantly, particularly at higher GFR [53].

1.10.3.1.1 Chronic Kidney Disease Epidemiology CKD EPI Equation

The CKD EPI creatinine was developed by continually modifying the creatinine-based equation in 2009, and it is preferred among other creatinine-based equations according to the Kidney Disease Improving global outcomes since it permits the reporting of stage 1 of GFR and its had low bias compared with other creatinine-based equations [54]. A constraint identified in the research is the restricted presence of racial and ethnic minorities; therefore, the CKD EPI creatinine update in 2021 is to be applied to certain other racial or ethnic groups without considering race [55].

However, these equations did not improve the clinical tracking of renal sickness sufficiently, even though since it was published, it has been regarded as the most accurate technique of estimating GFR, and hope with continued effort remains in finding an easy, inexpensive, and non-cumbersome method that is better and more accurate for calculating the glomerular filtration rate, since whatever formulas are used, differences in variables that affect blood creatinine restrict the accuracy of estimation glomerular filtration rate based on creatinine [34].

$$eGFR = 141 \times max\ (Scr\ /\ \kappa) - 1.209 \times min\ (Scr\ /\ \kappa)\alpha \times 0.993\ Age \times 1{:}018\ if\ female \times 1.159\ if\ Black. \quad 3$$

Whereas: Scr = standardized serum creatinine in mg/dL, κ = 0.7 (females) or 0.9 (males)

α = -0.241 (female) or -0.302 (male), min (Scr/κ, 1) is the minimum of Scr/κ or 1.0

max (Scr/κ, 1) is the maximum of Scr/κ or 1.0Cystatin C is a tiny molecular unit with a tertiary structure, categorized as a cystatin type 2. It was first identified in 1961 and given its official name in 1984 [56]. It is a non-glycosylated polypeptide, expressed by the CST3 gene, also known as cystatin 3, the most prevalent cystatin in humans, which is stably synthesized via all nucleated cells, freely filtered by the glomerulus, neither secreted nor reabsorbed [57]. Notably, cystatin C has a multifaceted role in the pathophysiology, whereas it is a significant regulator of extracellular cysteine protease inhibitors and lysosomal proteinases. In particular, it plays a critical role in regulating cathepsins-protease activity- inhibiting their action by binding the cysteine protease active site, which is overexpressed in lesions associated with atherosclerosis and aneurysms, an unbalanced functioning of protease and cystatins activity may be the basis of many pathologic conditions [56, 58]. Also, it indicates cardiovascular disease and renal transplant performance and could offer an improved approach to GFR estimation, according to the latest studies [59]. Cystatin c is primarily catabolized through the kidney, dramatically increasing once the kidney starts to impair. It has been demonstrated that the level of serum cystatin c is unaffected by some inflammatory diseases or other metabolic abnormalities, race, or age. It is unaffected by non-GFR dependents, and it was shown to be unrelated to lean body mass [60]. In contrast, serum creatinine has shown a significant association with lean body mass, suggesting that cystatin c may be used to assess renal function in individuals with varied body mass more accurately and offer a more accurate estimate of GFR than creatinine, especially in cases of initial kidney damage [61].

The revised guideline officially recognized the developmental process of cystatin C in chronic kidney disease diagnoses in 2013, where several studies have demonstrated that estimates of glomerular filtration rate based on cystatin C rather than creatinine are more accurate indicators of patients' healthcare [54]. Since running cystatin C is more expensive than running creatinine, research is ongoing into the fact that the practical implementation of this test is needed to improve the accuracy of clinical diagnosis of chronic kidney disease.

1.11 Comparison Between Creatinine and Cystatin C as eGFR Biomarker

Cystatin C may be a more accurate measure of GFR than serum creatinine. One explanation for this could be that muscle mass variations significantly impact serum creatinine but not serum cystatin C. The eGFR calculated by creatinine tends to be high in people with little muscle mass and low in people with high muscle mass; this is because creatinine is affected by body mass index, which decreases the validity of blood creatinine as a marker for GFR. Contrarily, serum cystatin C did not correlate with lean tissue mass and typically did not vary between males and females. In contrast, the creatinine level in the male was twenty percent greater than that of the female, and the average lean tissue mass of males was forty-two percent larger than that of women [62]. Cystatin C in eGFR significantly improved the calculation of GFR in hepatic dysfunction patients. Also, it had a substantially reduced bias compared to creatinine because liver cirrhosis causes approximately half a reduction in the synthesis of creatinine [48].

Also, serum cystatin C is more sensitive and has a higher diagnostic accuracy, specifically in people with slight declines in GFR. Identifying early renal insufficiency adds to the method of measuring cystatin C, which is not affected by serum turbidity as in hypertriglyceridemia, hemolysis, and hyperbilirubinemia compared to creatinine [63].

Studies have found a close link between chronic kidney disease and cardiovascular disease. These patients must use reliable indicators in GFR estimation to minimize misinterpretations of test findings and identify the proper drug dose by evaluating glomerular filtration rate (eGFR) based on creatinine and cystatin c markers in separate groups with heart disease. It noted a very stable discrepancy among both of the readings in patients treated at cardiology centres, where the average creatinine eGFR was about ten greater than the average cystatin C eGFR, which led to reclassified the proportion stages of patients based on the GFR marker used [64].

A study included middle-aged and older inhabitants in Sweden, where standard cystatin C analysis has been affordable for over a decade. Participants had serum creatinine and cystatin C levels measured at the same time. The result revealed that cystatin C-based eGFR was lower than creatinine-based eGFR in approximately half of the participants, and over five years of follow-up, those with the greatest negative difference in which- cystatin-based lower than creatinine-based eGFR – were found to had considerably increased odds of death, require renal replacement therapy, and have several cardiovascular outcomes than people with similar eGFR by the two methods [65]. An additional study analyzed data from a prior randomized trial that included almost two thousand patients' cystatin c and creatinine results. It found that one-third of patients with significantly lower eGFR-based cystatin c compared to eGFR-based creatinine had a significantly greater risk of cardiovascular-related death compared to patients with smaller variations [66].

In addition, in the case of steady renal function transplant patients, a study was conducted in 2005, where patients underwent 24-hour urine collection, weight and height measurements, and blood sampling to determine serum creatinine and cystatin C. The GFR was estimated using four equations based on serum cystatin C and those based on serum creatinine. GFR was determined using an exogenous marker as a reference value. According to measured GFR, every formula's bias, precision, and accuracy were verified, and it was demonstrated that cystatin C-based prediction equations are more reliable and accurate than the traditional creatinine-based equations at estimating GFR [67]. An estimation of serum creatinine and serum cystatin c were done to twenty-seven surviving renal donors, GFR was measured by using a diagnostic procedure in nuclear medicine that use gamma rays from radioactive isotopes to creates images of the body's internal organs and tissues, also renal function was estimated by the Cockcroft–Gault, MDRD, Chronic Kidney Disease Epidemiology -based creatinine equations, cystatin formulas, and creatinine–cystatin formulae, the finding showed that m-GFR had a poor relationship with Cys-c and creatinine equations alone, while investigation validates the use of creatinine - cystatin c equation for estimating GFR in healthy donors particularly those who are older and more prone to renal impairment following kidney donation, and according to this research it appears that cystatin c based formulae might serve better than those based on creatinine as stand-in instruments for following kidneys condition and evaluate GFR in normal aged renal donors, the CKD EPI cystatin formula has better specificity, whereas C–G equation has better sensitivity for determination of decline in renal filtration rate [68].

At the same time, a study conducted on one hundred and sixty-four individuals suffering from renal impairment diagnosed as G2-G3 chronic kidney disease underwent the exogenous golden method for determining GFR, concurrently serum levels of cystatin C and creatinine were tested in each subject to discriminate among mildly and moderately decreased in GFR, the overall biases were also computed by taking the mean differential among measured and estimated GFR values, and according to the finding, cystatin C was found to exhibit considerably better reliability for diagnosis and valid indicator in slight to intermediate glomerular filtration rate dysfunction compared to creatinine [51]. In a trial of kidney risk, about one thousand seven hundred forty-one elderly participants were selected and were diagnosed with stage 3 (G3a-G3b) chronic kidney disease. The GFR was estimated using CKD-EPI equations based on creatinine, cystatin C, and both. A significant proportion of patients with stage 3 CKD were reclassified using cystatin C. eGFR based on cystatin C or creatinine-cystatin C provided lower GFR estimates compared to creatinine-based eGFR. This affected most patients with stage 3 CKD. Re-diagnosis using cystatin C yielded different outcomes than creatinine. 7.7% of patients were reclassified as CKD-free. In contrast, 59% were categorized as having more severe CKD [69].

To evaluate the effectiveness of cystatin C and creatinine in estimating glomerular filtration rate, an additional study was conducted involving 184 individuals in good health as well as patients diagnosed with type II diabetes. The GFR was estimated based on the equation of creatinine and cystatin c. It was noted that the eighty-four diabetic participants included a greater percentage of males and, predictably, they were older and had a higher body mass index. It also noted that these patients had a reduced mGFR with greater readings in creatinine and cystatin c. The result showed that the CKD-EPI formula regarded people in good health possessed fewer biases and greater accuracy than the other group. In contrast, in the diabetic group, the GFR estimate was substantially understated compared to mGFR in every aspect of the formulas, and it showed remarkably increased biases for every aspect and much lower accuracy in this group [70].

$$\text{CKD EPI cystatin:} \quad eGFR = 133 \times \min\left(\frac{Scys}{0.8,1}\right)^{-0.499} \times \max\left(\frac{Scys}{0.8,1}\right)^{-1.328} \times 0.996^{Age} \times 0.932 \; if\ female. \qquad 4$$

$$\text{CKD EPI creatinine–cystatin:} \; eGFR = 135 \times \min\left(\frac{Scr}{\kappa},1\right)^{\alpha} \times \max\left(\frac{Scr}{\kappa},1\right)^{-0.601} x \min\left(\frac{Scys}{0.8},1\right)^{-0.375} x \max\left(\frac{Scys}{0.8},1\right)^{-0.711} x \qquad 5$$

$$0.995^{age} \; x \; 0.969 \; [if\ female] \times 1.08 \; if\ black \; .$$

Where κ is 0.7 for females and 0.9 for males, α is −0.248 for females and −0.207 for males [71].

A study aims to assess how well CKD-EPI creatinine-based and CKD-EPI cystatin C-based eGFR equations performed when reviewed to m-GFR, iothalamate elimination was used to calculate the glomerular filtration rate, creatinine, and cystatin C was tested. Also, the glomerular filtration rate was estimated by using formulas. The participants in this study included donors with good health, patients with chronic renal impairment, and organ transplantation patients. Generally, eGFR formulas appeared to understate mGFR. However, the comparison shows that highly reliable results were obtained with eGFRCr-Cys in various participants and had a low bias. Also, the cystatin-based formula alone had a lower bias than creatinine, excluding those who undergo transplantation, and these differences could be attributed to the immunosuppressive impact on cystatin c [72].

Research published in 2019 on several separated formulas to assess which is capable of predicting unfavorable nephritis prognosis following a heart operation, an analysis of eGFR was computed with these formulas on people over 18 before the heart operation in order to determine which of creatinine-based equations is most accurate in predicting after operation renal impairment among those undertaking heart surgery. The results revealed a substantial and robust correlation among the equations, particularly at reduced eGFR values. Nevertheless, the discrepancy among the equations increased with greater eGFR values. The results of multiple examines demonstrated a substantial correlation between a lower eGFR, as determined by each equation, and a higher risk of kidney injury following cardiovascular surgery. The eGFR computed using each equation was a reliable indicator of postoperative acute kidney injury. Also, it showed that individuals with eGFRs less than 60 ml/min/1.73 m2, by using all available equations, spent more time in the emergency room and were more likely to suffer from kidney injury after surgery. They also had greater rates of in-hospital mortality. In contrast, patients with discordant glomerular filtration rates had a worse outcome than those with a glomerular filtration rate of more than 60 mL/min/1.73 m2. As investigation demonstrated, the eGFR of the same patient varied considerably according to the equation used, and this is due to the variables on which the equations depend [73].

In summary, the findings of earlier research studies showed that cystatin C had greater diagnostic accuracy and sensitivity than creatinine in detecting the decline in GFR <60 ml/min. Also, cystatin c-based GFR equations may facilitate the estimation of GFR in patients with liver disease and individuals with varying levels of lean body mass, in addition to the ability to predict the risk of adverse renal and cardiovascular outcomes.

Chapter Two

Materials and Methods

2.1 Study Design

This study was conducted using a cross-sectional study, which provides data for describing the status of phenomena or relationships among phenomena-related variables at a fixed point in time.

2.1.1 Study Population

Participants in our study included individuals diagnosed and followed up at the nephrology outpatient clinic over the past year and newly diagnosed patients with chronic kidney disease, either stage 1 through 5, who were not undergoing dialysis. Both male and female participants aged 18-65 years were considered from Ramallah Governmental Hospital and Al-Watani Government Hospital in Nablus, as these hospitals house kidney clinics and specialists and are frequented by a large number of patients in Palestine.

2.1.2 Sample Size

The sample size was calculated based on the prevalence of CKD according to the Palestinian Central Bureau of Statistics (PCBS), and the prevalence was 20% in 2021.

The sample size was calculated using the following formula [74]:

$$n=(((Z)^{(2)} * P(1-P))/(d)^2 \quad n=((\,[1.96]^2 * 0.2\,(1-0.12))/((\,[0.05)]^2) \qquad (6)$$

$$n=(3.8416*0.2(0.8))/0.0025$$

n=245 participants

Where:

n = sample size, Z = value of 95% confidence interval (1.96), P = previous prevalence of CKD in Palestine = 0.2, d = margin of error (5%) = 0.05

2.1.3 Inclusion Criteria

Patients of both genders above 18 years were eligible to participate in the research provided they met the following criteria: Newly diagnosed patient with renal disease, patient with chronic disease; hypertension, diabetes mellitus and cardiovascular disease, recurrent urinary tract infection, recurrent stone, patient with renal disease family history where included.

2.1.4 Exclusion Criteria

Patients on dialysis, anuria patients who are waiting for dialysis, who have a history of malignancy, Pregnant women, patients with liver cirrhosis, thyroid dysfunction patients, and kidney transplant patients where excluded.

2.1.5 Ethical Considerations

Approval from the institutional review board (IRB) has been obtained from the An-Najah National University committee, in addition to approval consent from the General Directorate of Health Education and Scientific Research in the Ministry of Health to be approved for collecting samples from hospitalized patients. Also, Ramallah Hospital and Al-Watani Hospital approval was obtained before collecting samples. A consent form was obtained from each participant who agreed to participate, and a written questionnaire already prepared in Arabic was filled out by face-to-face interviews and patient files. The questionnaire questions were divided into two parts: the first asked about demographic variables like education level, employment, living area, and age, and the second asked about chronic disease related to general health, lifestyle, and family history with kidney disease.

2.2 Instruments of Study and Validation Indicators

The study was conducted under a cooperation agreement between the National Hospital in Nablus and Ramallah Governmental Hospital with An-Najah National University . After the approval was received from the Institutional Review Board, it was also obtained from the Palestinian Ministry of Health and government hospitals, where a kidney specialist is available. Informed consent was provided to the hospital's responsible persons, explaining the study's purpose and all the details required to be described. In addition to ensuring the confidentiality of the data, we have asked for a static of the number of patients in the early stages of chronic renal disease diagnosed in these hospitals in 2022–2023. Unfortunately, there was a problem with the accuracy of the statistics, as the diagnosis was inaccurate, so we entered the hospital system and selected our patients according to inclusion and exclusion criteria.

Then, the case report form was filled out by collecting the patient information from the patient files with the help of specialist doctors, and a discussion was conducted with the patients themselves. All participants underwent a complete history taking and comprehensive clinical examination. Blood samples were collected according to standard laboratory routines from the patients who met the search criteria in a plan tube. Five ml of blood was taken at complete rest, samples were drawn early to reduce dietary and diurnal changes, and then it was transferred to the hospital laboratory for creatinine analysis and stored at -70ºC or at -80ºC until cystatin c was measured. Each sample was tested for cystatin c and creatinine at duplicate.

2.2.1 Data Collection

Patients who fit the search criteria were determined by dealing with the outpatient clinics for renal illness. Clinical information about patients was collected using patient records from hospitals and packaged in case report forms for efficient and complete data collection, processing, and analysis. The case report form included the patient registration number, gender, and age. Additionally, it also has other details regarding the patient’s medical history, such as if they have diabetes, hypertension, thyroid dysfunction, liver diseases, history of cancer, or recurrent UTI. A family history of CKD, the patient's initial diagnosis, and the patient's stage of illness are also included. The method of discovering the condition, whether by chance, routine testing, or due to the deterioration of the condition. Some questions about whether the patient is used to taking energy drinks and painkillers and whether he is an athlete.

Patients who fit the study criteria decided if they wanted to participate by perusing the informed consent, which includes defining the name and purpose of the research, describing the risks and inconveniences that the participant may be exposed to, and a location to sign the consent.

The informed consent formula states that we are An-Najah University, clinical biochemistry master's students. We want to conduct a study on a technique for early detection of chronic kidney disease. We kindly ask you to assist us by taking part in the study, in which you will be required to give a blood sample while being aware that you won't experience anything more than a needle poke as a result of your participation without any risk to your health. Your participation in the study will be completely optional, and all of your personal information will be kept completely secret and used exclusively for the intended purposes of the study.

The sample was collected from a plane tube in Ramallah and Al-Watani hospitals and then separated to obtain the serum and transfer it with an ice box to the Palestinian Ministry of Health in Beddia, where the testing will be conducted due to the presence of cystatin c device there.

2.2.2 Cystatin C Test

Cystatin C was analyzed in the Ministry of Health in Beddia by particle-enhanced immunoturbidimetric method, which is used to determine the amount of antigen by applying the principle of light turbidimetric assay to antigen-antibody reaction. Cystatin C concentration is determined by photometric measurement of antigen-antibody reaction between antibodies against cystatin C bound to polystyrene particles and cystatin C present in the sample. Calibration and control were done before analysis. The test was done in duplicate.

Reduced glomerular filtration rate and, consequently, renal failure are associated with elevated blood levels of cystatin C. Cystatin C should stay at a consistent level in the blood because it is created continuously throughout the body and eliminated and broken down by the kidneys. As long as the GFR is normal and the kidneys are functioning well.

Cystatin C concentrations in subjects with normal GFR ranged from

19-49 years: 0.53 – 0.92 mg/L

>50 years: 0.58 – 1.02 mg/L

GFR was estimated using the CKD EPI-based cystatin C.

2.2.3 Creatinine Test

Serum creatinine was measured in the hospital using an enzymatic method that utilizes a multi-step approach, ending with a photometric endpoint reaction. The reagent, ready to use, is composed of amidohydrolase, which is used to convert creatinine to creatine, which in turn catabolized to sarcosine and urea by the amidohydrolase enzyme, and the sarcosine oxidase, which produces a colored chromogen which Budget and Justification

The total grant for the MSC thesis is 1000 $ distributed as follows

- Mainly cystatin c: Each kit cost about (295) $, the calibrator cost (355) $, the control cost (145) $, and each kit can do the test for about 80 patients.
- Mainly creatinine: Each kit cost about (109) $. The kit contains a calibrator, and each kit can do the test for about 200 patients.

2.3 Statistical Analysis

The Statistical Package for Social Science (SPSS), version 26, was used for data entry and analysis. A significant test (P-value) tested the hypothesis. The P-value was less than 0.05, which was considered to indicate statistical significance, and the null hypothesis was rejected (Cystatin C is a better predictor marker of GFR in an early stage of CKD). Descriptive statistical analysis was done for age, creatinine, and cystatin c kidney function markers, and CKD-EPI estimated glomerular filtration rates (eGFR). The CKD-EPI eGFR equations were used to calculate the population distribution of CKD stages. The relationships between various CKD-EPI equations are displayed using Spearman's rho (Non-Parametric relationships). Regression analyses aim to determine these equations' predictive power and reliability in estimating kidney function in CKD patients. The Chi-square analysis evaluated the association between different CKD staging methods.

Chapter Three

Results

3.1 Introduction

This chapter presents the research results of the patients diagnosed with chronic kidney disease (CKD). It contains the answers to the questionnaires filled out by the participants, which include demographic data, previous medical history, lifestyle, protein intake, as well as the how the disease was diagnosed. Moreover, the chapter encompasses the kidney performance measurements employing creatinine and cystatin c markers. The normality tests, regression analysis, and the correlations of the various CKD-EPI equations are also addressed. The relationships between various equations for estimating the glomerular filtration rate (eGFR) within the designated CKD eGFR range were then compared using chi-square analysis.

3.2 Patient's characteristics

The study included 246 individuals diagnosed with CKD, as 27 individuals were excluded due to the deterioration of their health condition and their recent exposure to dialysis, 39 of them refused to participate, and 19 pregnant females were excluded from the study. Of the 160 patients who were diagnosed with CKD, 23.1% were in the first stage, 13.1% were in the second stage, 40% were in stage 3, and 18.8% were in stage 4. The population distribution was 55% male and 45% female, with a mean age of 46 years old.

3.2.1 Sociodemographic characteristics

Table 2 presents the sociodemographic characteristics of the 160 participants, including their age, duration of diabetes (DM), and duration of hypertension (HTN). The ages ranged from 18 - 65 years, with an average of 46 years. The duration of diabetes varied from non-diabetic to 30 years, with an average of 5 years. Similarly, the duration of hypertension ranged from 0 to 25 years, with an average of approximately 4 years.

Table 2

Sociodemographic characteristics of the patients

Scale Variables	Minimum	Maximum	Mean	Std. Deviation
Age	18	65	46.34	14.257
Duration of HTN	0	25	3.97	5.416
Duration of DM	0	30	5.44	6.132

3.2.2 Medical history information

Table 3 provides data on the participant's medical history, including the prevalence of various chronic diseases, cardiovascular conditions, urinary tract diseases, autoimmune diseases, and the family history of kidney issues among 160 participants. The information was gathered from computerized hospital records.

Table 3

Medical history information

Medical History Patient		Frequency	Percent
Diabetes	No	81	50.6
	yes	79	49.4
Hypertension	No	69	43.1
	yes	91	56.9
Cardiovascular disease	no	129	80.6
	yes	31	19.4
Recurrent urinary tract infection	no	119	74.4
	yes	41	25.6
Kidney stone	no	130	81.3
	yes	30	18.8
Auto immune disease	no	127	79.4
	yes	33	20.6
Lower urinary tract blockage	no	152	95.0
	yes	8	5.0
Reduction in kidney mass	no	149	93.1
	yes	11	6.9
Family history of kidney disease	no	126	78.8
	yes	34	21.3

3.2.3 Lifestyle habits

Table 4 displays the habits of 160 participants via a questionnaire. The participants were asked whether they had a sedentary life or practiced any type of physical activity, even walking. Approximately 52.5% of the individuals reported engaging in physical activities, whereas 47.5% had a sedentary lifestyle. Additionally, 33.1% of the participants regularly used painkillers, particularly nonsteroidal anti-inflammatory drugs (NSAIDs). Also, most participants met their daily water intake requirements, with approximately 2.5 L for adult males and 2 L for adult females, respectively, total water and 1.5 of daily water intake [75, 76].

Table 4

Participant lifestyle habits.

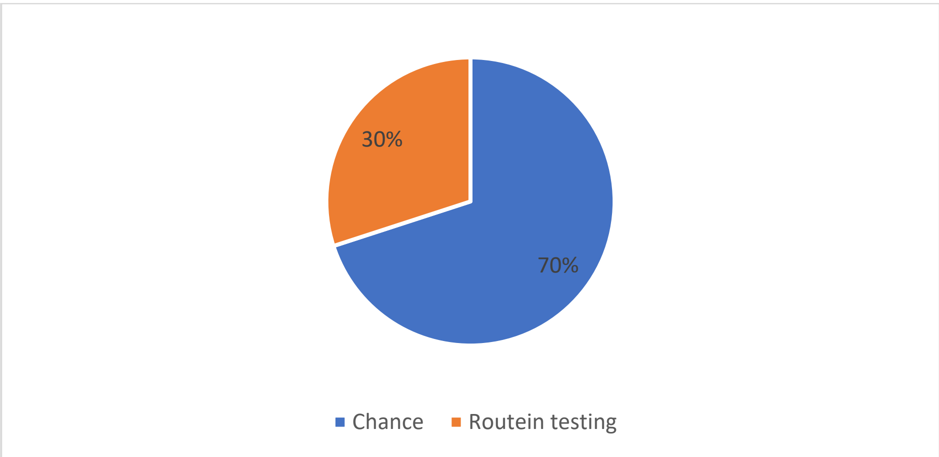
Life Style habits		Frequency	Percent
Sport	No	76	47.5
	Yes	84	52.5
Pain Killer	No	107	66.9
	Yes	53	33.1
Energy Drink	No	134	83.8
	Yes	26	16.3
Enough Water	No	23	14.4
	Yes	137	85.6

3.2.4 Disease discovery

Figure 1 illustrates how participants discovered their chronic kidney disease. The majority, 70%, reported their condition by chance. At the same time, 30% discovered it through routine testing.

Figure 2

Percent of the way the disease was discovered



3.3 Stages of chronic kidney disease based on CKD-EPI eGFR equations

Figure 2 shows the distribution and concordance of CKD stages among three staging equations: CKD-EPI eGFR based on creatinine, CKD-EPI eGFR-based Cr-C equation 2021, and CKD-EPI eGFR-based Cyst c equation 2012. Key findings include the following:

- eGFR based on creatinine level: Most patients were classified into stage 1 (23.1%) and stage 3b (20.6%), with the fewest being classified into stage 5 (5.0%).
- CKD-EPI eGFR-based Cr-C equation 2021: The highest frequencies were observed in stage 4 (22.5%) and stage 3b (21.9%), whereas the lowest was in stage 5 (6.3%).
- CKD-EPI eGFR-based Cyst c equation 2012: This method showed the highest frequency in stage 3b (25.0%) and stage 4 (22.5%) patients, with the lowest frequency in stage 2 (12.5%) and stage 3a (12.5%) patients.

These results demonstrate significant agreement between the CKD-EPI eGFR-based Cr-C equation and the CKD-EPI eGFR-based Cyst c equation 2012, with minor discrepancies in stage classification. The distribution percentages highlight the reliability of the CKD-EPIDistribution of kidney disease stages based on CKD EPI eGFR equations

3.4 Biochemical markers and CKD-EPI equations

Table 6 presents descriptive statistics for age, renal biochemical markers, and CKD-EPI estimated glomerular filtration rates (eGFR) using creatinine and cystatin c markers.

Table 5

Patients result for creatinine(mg/dl), cystatin c (mg/l) and CKD-EPI equations (mL/min/1.73 m2)

	Minimum	Maximum	Median	Mean	Std. Deviation
Age	18.00	65.00	50.0000	46.3375	14.25724
Creatinine value (mg/dl)	.40	7.69	1.5000	1.8755	1.29711
Cystatin C value (mg/l)	.46	4.86	1.7000	1.8659	.97436
CKD-EPI eGFRCr equation	7.00	147.00	50.0000	58.7313	36.43409
CKD-EPI eGFRCr-C equation	8.00	138.00	44.0000	54.9500	35.90971
CKD-EPI eGFR Cys c equation	10.00	136.00	39.0000	51.3250	34.10427

CKD-EPI: chronic kidney disease epidemiology, eGFRCr: estimated glomerular filtration rate-based creatinine, eGFRCr-C: estimated glomerular filtration rate-based creatinine and cystatin c, eGFR Cys: estimated glomerular filtration rate-based cystatin.

3.5 Normality test

Table 7 displays the Kolmogorov-Smirnov and Shapiro-Wilk test results, which indicate significant deviations from normality for all the variables.

Table 6

Normality test

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	Df	Sig.	Statistic	Df	Sig.
Age	.130	160	.000	.920	160	.000
Creatinine value (mg/dl)	.173	160	.000	.830	160	.000
Cystatin c value1(mg/l)	.114	160	.000	.924	160	.000
CKD-EPI eGFRCr equation (mL/min/1.73m2)	.144	160	.000	.916	160	.000
CKD-EPI eGFRCr-C equation (mL/min/1.73m2)	.147	160	.000	.896	160	.000
CKD-EPI eGFR Cys c equation (mL/min/1.73m2)	.180	160	.000	.883	160	.000

3.5.1 Correlation between the equations

Table 8 shows the Spearman's rank correlation coefficients between the three CKD-EPI eGFR equations. Strong positive correlations among all pairs of variables indicate a high degree of association. The correlation coefficient between the equation based on creatinine and the equation based on both creatinine and cystatin c was 0.974 ($p < 0.001$), indicating a very high degree of association between the results obtained from these two equations. Similarly, the correlation coefficient between the equation based on creatinine and the equation based on cystatin c was .929 ($p < .001$), and that between the equation based on both creatinine and cystatin c and the equation based on cystatin c was 0.986 ($p < 0.001$), demonstrating very strong correlations as well. These results imply a consistent and robust relationship between the variables, suggesting that regardless of the specific equation used, the results tend to align closely with those obtained from other equations. Moreover, the significant p-values associated with these correlations indicate that the observed associations are unlikely to have occurred by chance alone.

We can assess the correlation coefficients to determine which equation is the closest to the 2021 CKD-EPI eGFRCr equation. The CKD-EPI equation, which is based on both creatinine and cystatin c, has the highest correlation coefficient with the CKD-EPI equation, which is based on the creatinine, at .974 ($p < .001$), indicating an extremely strong relationship. While the correlation coefficient between the CKD-EPI -based creatinine equation and the CKD-EPI- based cystatin c equation was also high at 0.929 ($p < 0.001$), it was slightly lower than the correlation coefficient between the CKD-EPI-based both creatinine and the cystatin c equation. Therefore, on the basis of the correlation analysis, the CKD-EPI eGFRCr-C equation appears to be the equation closest to the CKD-EPI eGFRCr equation.

Table 7

Non-parametric correlations of the CKD-EPI eGFR equations

		CKD-EPI eGFRCr equation	CKD-EPI eGFRCr-C equation	CKD-EPI eGFR Cys c equation
CKD-EPI eGFRCr equation	Correlation Coefficient	1.000	.974**	.929**
	Sig. (2-tailed)		.000	.000
CKD-EPI eGFRCr-Cys equation	Correlation Coefficient	.974**	1.000	.986**
	Sig. (2-tailed)	.000		.000
CKD-EPI eGFR Cys equation	Correlation Coefficient	.929**	.986**	1.000
	Sig. (2-tailed)	.000	.000	

3.6 Regression Analysis with R-Squared: Detailed Comparison and Discussion

This analysis was conducted in this study to examine the interrelationships between the various CKD-EPI eGFR equations. The analysis aims to determine these equations' predictive power and reliability in estimating kidney function in CKD patients. Two basic regression models were assessed: model 1 compared the CKD-EPI eGFRCr equation to the CKD-EPI eGFRCr-C equation, and the other model compared the CKD-EPI eGFRCr equation to the CKD-EPI eGFR-based Cyst c equation.

3.6.1 Model 1: CKD-EPI eGFRCr equation vs. CKD-EPI eGFRCr-C equation

The first regression model was examines the relationship between the CKD-EPI eGFRCr equation (dependent variable) and the CKD-EPI eGFRCr-C equation (independent variable).

Table 8

CKD-EPI eGFRCr equation vs. CKD-EPI eGFRCr-C equation regression analysis

Variable	Coefficient	Standard Error	t-value	p-value	95% Confidence Interval
Intercept	4.985	1.0405	4.79	0.000	2.930381 - 7.040836
CKD-EPI eGFRCr-Cys	0.978	0.02463	39.70	0.000	0.929428 - 1.026737
R-squared	0.929				

3.6.2 Model 2: CKD-EPI eGFRCr equation vs. CKD-EPI eGFR cyst c equation

The second regression model examines the relationship between the CKD-EPI eGFRCr equation (dependent variable) and the CKD-EPI eGFR cyst c equation (independent variable).

Table 9

CKD-EPI eGFRCr equation vs. CKD-EPI eGFR cyst c equation regression analysis

Variable	Coefficient	Standard Error	t-value	p-value	95% Confidence Interval
Intercept	8.803	1.8	4.89	0.000	.892 - 1.053
CKD-EPI eGFR Cyst C	0.9727	0.04	23.89	0.000	5.244 - 12.361
R-squared	0.8292				

3.6.3 R-square (R^2)

R-squared values indicate the proportion of variance in the dependent variable that the independent variable can explain. Higher R-squared values suggest a better model fit.

- Model 1 (CKD-EPI eGFRCr vs. CKD-EPI eGFRCr-C): R-squared = 0.929
- Model 2 (CKD-EPI eGFRCr vs. CKD-EPI eGFR cyst c): R-squared = 0.8292

The higher R-squared value in Model 1 indicates a better fit than that in Model 2. This means that the CKD-EPI eGFRCr-C equation explains more of the variance in the CKD-EPI eGFRCr equation than does the CKD-EPI eGFR cyst c equation.

3.6.4 Coefficients (Coef.)

Both models demonstrated high coefficients close to 1, indicating a strong linear relationship between the CKD-EPI eGFRCr equation and the other two equations.

- Model 1 (CKD-EPI eGFRCr vs. CKD-EPI eGFRCr-C): The coefficient is 0.978, suggesting a nearly one-to-one correspondence between the two equations.
- Model 2 (CKD-EPI eGFRCr vs. CKD-EPI eGFR cyst c): The coefficient is 0.9727, which also indicates a strong linear relationship.

3.6.5 Standard errors (Std. Err.)

The standard errors for the coefficients in both models are relatively low, indicating precise estimates. The slightly higher standard error in Model 2 (0.04) suggests marginally less precision than in Model 1 (0.02463).

3.6.5.1 t-values (t) and p-values (P>|t|)

Both models have extremely high t-values and significant p-values ($p < 0.001$), indicating that the relationships between the equations are highly statistically significant. Since the t value in Model 1 = 39.70, $p < 0.001$, where the t value in Model 2 = 32.41, $p < 0.001$, the higher t-value in Model 1 further supports the stronger relationship between the CKD-EPI eGFRCr equation and the CKD-EPI eGFRCr-C equation.

3.6.6 Interpretation and Clinical Relevance

The regression analysis indicated that both the CKD-EPI eGFRCr-C equation and the CKD-EPI eGFR cyst c equation are highly reliable for estimating kidney function compared with the CKD-EPI eGFRCr equation. However, the stronger correlation (as indicated by the coefficient) and the higher R-squared value in Model 1 suggest that the CKD-EPI eGFRCr-Cyst c equation might be a marginally better predictor of eGFR in this patient cohort.

These findings are crucial for clinical practice as they provide evidence of the reliability of different eGFR estimation methods, which can guide healthcare professionals in selecting the most appropriate equation for assessing kidney function in CKD patients. The high degree of correlation among these equations confirms their utility in various clinical settings, ensuring accurate monitoring and management of CKD.

3.6.7 Normality test of residuals

The Shapiro-Wilk W test was conducted to assess the normality of the residuals. Since the p-value is greater than the common significance level of 0.05, we fail to reject the null hypothesis that the residuals are normally distributed. Consequently, no significant evidence suggests that the residuals deviate from a normal distribution.

Table 10

Shapiro-Wilk W Test for Normal Distributed Data

	Statistic	Sig.
CKD-EPI eGFRCr vs. CKD-EPI eGFRCr-C	-1.0364334	0.15
CKD-EPI eGFRCr vs. CKD-EPI eGFR Cyst C	-0.77219321	0.22

3.7 Concordance of CKD staging

Based on figures 4, 5, and 6, the crosstabulation analyses present the concordance of CKD stages based on different staging equations: the eGFR calculated via the creatinine equation versus the CKD-EPI eGFRCr-C equation, the eGFR calculated via the creatinine equation versus the CKD-EPI eGFR cyst c equation, and the CKD-EPI eGFRCr-C equation versus the CKD-EPI eGFR Cys C equation. The findings indicate significant levels of agreement across the staging methods. Specifically, 26 out of 37 patients classified as stage 1 according to the eGFR based on the creatinine level were classified as stage 1 according to the CKD-EPI eGFR-based cyst c equation. Similarly, 32 out of 37 patients classified as stage 1 by eGFR based on creatinine were also classified as stage 1 by the CKD-EPI eGFRCr-C equation. The concordance rates for the other stages also showed substantial agreement, although some discrepancies were noted, particularly in stages 2 and 3a.

Based on the comparison between the 2021 CKD-EPI eGFRCr-C equation and the 2012 CKD-EPI eGFR cyst C equation, a high level of concordance was observed since both equations classified 28 out of 34 patients as stage 1. For stages 3b and 4, the concordance rates are 28 out of 35 and 30 out of 36, respectively. This indicates that despite a small variation, CKD-EPI eGFR generally agrees on the CKD stages. These results suggest that the equation, which utilizes both creatinine and cystatin c markers appears to be more valid compared with eGFR-based cystatin c and eGFR-based creatinine each separately, thereby supporting their use in clinical practice.

Figure 3

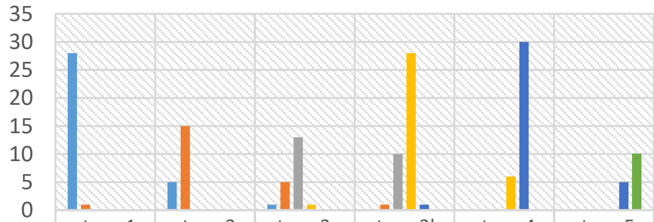
CKD EPI eGFR Creatinine versus CKD EPI eGFR Cystatin C Cross-tabulation

	stage 1	stage 2	stage 3a	stage 3b	stage 4	stage 5
	eGFR based creatinine					
CKD EPI eGFRCr-cyst c stage 1	32	2	0	0	0	0
CKD EPI eGFRCr-cyst c stage 2	5	14	3	0	0	0
CKD EPI eGFRCr-cyst c stage 3a	0	4	18	1	0	0
CKD EPI eGFRCr-cyst c stage 3b	0	1	9	24	1	0
CKD EPI eGFRCr-cyst c stage 4	0	0	1	8	26	1
CKD EPI eGFRCr-cyst c stage 5	0	0	0	0	3	7

CKD EPI eGFRCr-cyst c stage 1 CKD EPI eGFRCr-cyst c stage 2
CKD EPI eGFRCr-cyst c stage 3a CKD EPI eGFRCr-cyst c stage 3b
CKD EPI eGFRCr-cyst c stage 4 CKD EPI eGFRCr-cyst c stage 5

Figure 4

CKD EPI eGFR Creatinine versus CKD EPI eGFR Creatinine -Cystatin C Cross-tabulation



	stage 1	stage 2	stage 3a	stage 3b	stage 4	stage 5
	CKD EPI eGFR cystatin c					
CKD EPI eGFRCr-cyst c stage 1	28	5	1	0	0	0
CKD EPI eGFRCr-cyst c stage 2	1	15	5	1	0	0
CKD EPI eGFRCr-cyst c stage 3a	0	0	13	10	0	0
CKD EPI eGFRCr-cyst c stage 3b	0	0	1	28	6	0
CKD EPI eGFRCr-cyst c stage 4	0	0	0	1	30	5
CKD EPI eGFRCr-cyst c stage 5	0	0	0	0	0	10

CKD EPI eGFRCr-cyst c stage 1 CKD EPI eGFRCr-cyst c stage 2
CKD EPI eGFRCr-cyst c stage 3a CKD EPI eGFRCr-cyst c stage 3b
CKD EPI eGFRCr-cyst c stage 4 CKD EPI eGFRCr-cyst c stage 5

3.8 Chi-square test: Statistical analysis of staging categories in chronic kidney disease

In this study, we used a chi-square test to compare the associations between eGFR equations based on creatinine ,cystatin c and both creatinine and cystatin c derived from CKD-EPI 2021 equations. The test specific objective is to determine whether the distributions of these eGFR equations are independent of each others or if they are significantly correlated. This investigation highlights the dependability and consistency of these equations across various eGFR protocols.

3.8.1 Interpretation of the Results in Table 12

3.8.1.1 CKD-EPI eGFRCr * CKD-EPI eGFR Cyst C

The analysis revealed a statistically significant relationship between eGFR based creatinine and eGFR based cystatin c. As indicated by a chi-square value of 284.376 on 25 degrees of freedom and p-value of 0.000. These results reveal that there is a considerable correlation between the eGFR values obtained from creatinine and those obtained from cystatin c, suggesting that the distributions of these two equations are not independent.

3.8.1.2 CKD-EPI eGFRCr * CKD-EPI eGFRCr-cyst C

The findings reflect that there is a considerable relationship between the two equations CKD-EPI eGFRCr and CKD-EPI eGFRCr-cyst C, as indicated by the chi-square value of 428.631 with 25 degrees of freedom and a p-value of 0.000.

3.8.1.3 CKD-EPI eGFRCr-cyst C * CKD-EPI eGFR Cyst C

The chi-square value of 460.551 with 25 degrees of freedom and a p-value of 0.000 revealed a highly significant association between the combined creatinine-cystatin c equation (eGFRCr-cyst c) and the cystatin c-based equation (eGFR Cyst c). This finding suggests that the eGFR values from the combined method are significantly associated with those from the cystatin c-based method.

3.8.2 Comparison of the results

3.8.2.1 Significance Levels

All three chi-square tests exhibit extremely low p-values ($p < 0.0001$), indicating strong evidence against the null hypothesis of independence. This means a significant association exists between each pair of eGFR equations.

3.8.2.2 Strength of Association

The chi-square values varied among the pairs, with the highest value (460.551) observed between the combined equation (eGFRCr-cyst c) and the cystatin c-based equation (eGFR cyst c). This suggests that the association is strongest between these two methods.

The second-highest chi-square value (428.631) was observed between the creatinine-based equation (eGFRCr) and the combined equation (eGFRCr-cyst c), indicating a strong association. The associations between the creatinine-based equation (eGFRCr) and the cystatin C-based equation (eGFR Cyst c) are also significant but relatively lower (chi-square value of 284.376) than those of the other pairs.

The chi-square test results revealed significant associations between the different CKD-EPI eGFR equations. The strongest association was observed between the combined creatinine-cystatin c equation and the cystatin c-based equation, followed by the creatinine-based and combined equation. These findings suggest that while each equation provides valuable information, there is considerable overlap in the eGFRs they produce, indicating that they are not entirely independent. This information is crucial for clinicians and researchers in selecting the most appropriate eGFR estimation method for different patient populations.

3.9 Conclusion

The presented results show that cystatin c-based equations (both alone and combined with creatinine) have strong correlations, high predictive power, and significant associations with the creatinine-based equation. The CKD-EPI eGFRCr-Cyst C equation, in particular, demonstrated better performance in regression analysis, suggesting its superiority as a predictor of the GFR.

Chapter Four

Discussion

The primary aim of the present study is to determine the performance of cystatin C as a biomarker to estimate the glomerular filtration rate (GFR) in chronic kidney disease (CKD) patients aged 18-65 years in Palestine, and compare the accuracy of both cystatin C and creatinine. Our findings indicate a strong correlation in both creatinine and cystatin c dependents eGFR, however cystatin C is a superior marker of GFR in the early stages of CKD where conventional methods of measuring GFR are less sensitive. This is in line with the theory that cystatin C is a better predictor of GFR than creatinine.

This age group was chosen because chronic kidney disease (CKD) is prevalent in the aged people and, even in moderate to severe cases the majority of older patients, particularly those 70 years of age and older, had a much higher risk of dying than developing ESRD. On the other hand, younger patients who had significant or mild eGFR reductions had a much higher chance of developing ESRD than of passing away. Furturemore the physiological age-related fall in GFR becomes more noticeable in most older patients [78].

4.1 Interpretation

Cystatin C is a small protein which is freely filtered in the glomeruli before being reabsorbed and processed by the tubules [59]. Consequently, it is a potential candidate for a new GFR estimating biomarker[56]. At present, GFR is most commonly estimated using serum creatinine, a biomarker with both limited sensitivity and specificity for detection of renal impairment. In addition, as GFR decreases, the sensitivity of serum creatinine is significantly reduced, making early identification of renal impairment more challenging [79].

Cystatin C can help overcome such challenges by giving a more constant and accurate measure of kidney function[59]. Although there is no universal agreement about the usual cut-off point of cystatin C level to categorize patients as having CKD, several studies revealed that this diagnostic test is superior to serum creatinine, especially in particular populations and clinical settings [80].

Our results demonstrated that cystatin C-based eGFR equations consistently provided lower GFR estimates than creatinine-based equations, particularly in patients with early renal impairment. This finding is consistent with previous research suggesting that cystatin C is less influenced by non-GFR-related factors such as muscle mass, diet, and age, which often affect creatinine levels [63]. Furthermore, the high sensitivity of cystatin C in detecting mild decreases in GFR highlights its potential for early CKD diagnosis and management [51]. Additionally, it revealed strong correlations between the different CKD-EPI eGFR equations, particularly the combined creatinine-cystatin C equation (eGFRCr-cyst c) and the cystatin C-based equation (eGFR cyst c), suggests that using both biomarkers together may provide a more accurate estimation of GFR. The CKD-EPI equation combining cystatin C and creatinine (eGFRCr-cyst C) has strong predictive power for assessing kidney function and provides a more accurate GFR estimate than using either biomarker alone. This aligns with White et al. (2005), who showed that combined biomarker approaches yield more precise GFR estimations, especially in kidney transplant patients [67]. Similarly, Carrero et al. (2023) found that discrepancies between creatinine and cystatin C-based GFR estimates could be mitigated using a combined approach [81]. This is supported by the high correlations observed in our study between eGFRCr-cyst C and eGFR cyst c equations.

Our finding is consistent with previous research highlighting the clinical utility of cystatin C as a reliable and stable biomarker for GFR estimation. Cystatin C-based equations offer superior diagnostic accuracy than creatinine-based approaches, improving cystatin C's widespread implementation in clinical practice[64]. Similar to our study, Stevens et al. used the CKD-EPI equation and found that cystatin C-based eGFR estimates were more accurate in detecting early-stage CKD compared to creatinine-based estimates [82]. This is particularly relevant for our study, as early detection of CKD is crucial for improving patient outcomes. Both studies highlight cystatin C's importance in improving CKD diagnostic accuracy.

In comparison with Inker et al. (2012), our study aligns with Inker et al., who validated the CKD-EPI cystatin C equation and found that it performed better than creatinine-based equations in estimating GFR across different populations. Both studies emphasize the utility of cystatin C in clinical practice [71].

4.1.1 Correlation and regression analysis

The strong associations observed between the different GFR equations in our study are consistent with the results of previous studies that also reported high associations between creatinine- and cystatin C-based GFR estimates [71, 83]. These studies used regression analysis to compare the predictive power of the different GFR equations and consistently found that cystatin C-based equations provided better accuracy and less bias.

4.1.2 Specific comparisons

Our study sample was Palestinians, however, contributes a distinct viewpoint to the global knowledge on CKD biomarkers. Our study population's demographic characteristics and lifestyle factors are notably different from those found in many Western studies. The higher prevalence of comorbid conditions such as hypertension and diabetes in our cohort was similar to that from the Arab world, but higher than that usually seen in Western populations. This highlights the applicability of our findings to regional healthcare issues

In addition, our study was conducted in a resource-limited setting, and we highlight the practical implicatiPredictive ability for mortality and heart failure

Several studies have pointed to the conclusion that cystatin c is a better predictor of death and heart failure compared to the other biomarker creatinine. Cystatin c-based eGFR was a more reliable indicator of adverse outcomes; in previous studies the risk of cardiovascular disease also kidney disease inorder to elevated risk of mortality were positively associated with cystatin C elevated especially in female and aged people[84]. According to a different study, patients with normal creatinine levels and high levels of cystatin C were at greater risk of heart failure and mortality[85]. Our findings support these studies and and suggest that there might be improved risk stratification and follow-up of the CKD patients if the routine test employed were to include cystatin C.

4.1.3 Reclassification of participants' CKD stages

espite expectation that the classification of CKD based on eGFR is going to remain essential, advancements in CKD patient diagnosis and risk assessment are crucial. It is rarely possible to assess GFR at routine setting in Palestine using an exogenous GFR marker. Thus, low-cost endogenous GFR markers like creatinine and cystatin C will be used ongoing.

The changes in CKD stages due to other biomarker examination resulted in significant clinical implications. In our finding, some participants were reclassified after assessment based on cystatin c comparative to creatinine assessment alone. This concurs with results coming from studies which explained that inclusion of cystatin C in estimating eGFR changes significantly the assignment of CKD stages. This reclassification is particularly important as it can influence treatment approaches and prognosis. For instances to prevent future progression into renal failure, patients with cystatin c-classified CKD at more advanced stages may get more protocols, therapy choices and management strategies.

4.2 Implications

The findings have several essential implications for practicing and research in nephrology clinical practice.

- Advanced diagnostic techniques: The inclusion of cystatin c enhances the capacity of seeking diagnosis to detect early renal impairment during the assessment of chronic kidney disease. This could result in active management which might impair the course of the disease enhancing the patients' prognosis.
- Management of patients: The use of cystatin c routine practice in the clinics may serve as a good parameter for making clinical decisions like dosage and monitoring disease progress and reducing the creatinine affected result. This is so more so in the case of patients with other medical problems that decrease their muscle mass like the elderly and the malnourished.
- Resource allocation: Though the cost of performing uncovering cystatin C is higher than that of creatinine,its stable un like creatinine reagent and its benefit of enhanced performance is likely to be appropriate in certain patient groups thereby guiding resource allocation in the healthcare industry

4.3 Limitations

The limitation of this study :

1. Sample size and population: The study was done with a relatively small population in Palestine. Future research should involve a bigger sample size represetative population to improve the generalizability of the findings.
2. cost considerations: The increased expense of cystatin C testing remains a barrier to prevent its broad use. Cost-effectiveness analyses should be investigated in future research to have a better understanding of the financial effects of implementing cystatin C testing into routine clinical practice.
3. Longitudinal data: This cross-sectional study evaluate kidney function at one time point. Longitudinal studies are needed for future research to determine the effect of cystatin C on CKD progression and outcomes.
4. Lack of data documentation: Lack of documentation of results and diagnosis in the government hospital program, led to the inability to access the necessary data, and affect the interpretation of these results inaddition to this fundamentally affects statistics

4.4 Recommendations

Based on our findings, we recommend:

- Clinical Implementation: Providers should implement routine cystatin C assessment in addition to creatinine in the management of their high risk CKD patients.
- Further research: To examine the long-term advantages and economic viability of cystatin c testing, more studies with larger populations and longer time durations are required.
- Education and training: Clinicians should be educated and trained on the use as biomarker of eGFR.

4.5 Conclusion

There is great convergence in GFR diagnosis using both markers; however, there were few differences in some cases, with stability in the value by using cystatin c marker. Our results confirm that cystatin C is a useful marker of GFR for diagnosing CKD with better, stability compared to creatinine. Improving patients care and outcomes by increasing the accuracy in diagnosis CKD and allowing for earlier detection of renal impairment particulary in some cases that where the creatinine result fluctuates due to influences.The role of cystatin C testing in improving the management of patients with chronic kidney disease will be clearer when future studies are completed and when cystatin C testing enters clinical practice.

]List of Abbreviations

Abbreviation	Meaning
CKD	Chronic kidney disease
eGFR	Estimated glomerular filtration rate
ESRD	End stage renal disease
MDRD	Modification of Diet in Renal Disease
CKD EPI	Chronic Kidney Disease Epidemiology
Scr	serum creatinine
CKD-EPI eGFR-based Cr-C	Chronic Kidney Disease Epidemiology based creatinine and cystatin c
Cyst c	cystatin c

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Appendices

Appendix A

Consent Form

Informed consent

Study Title: Implementing Cystatin C as a biomarker to improve diagnostic accuracy in chronic kidney dysfunction in Palestine

A group of researchers at An-Najah National University, Faculty of Medicine (Nablus, Palestine), are conducting a study on the chronic kidney disease and the biomarkers to diagnose the disease. We kindly ask you to assist us by taking part in the study, in which you will be required to give a blood sample while being aware that you won't experience anything more than a needle poke as a result of your participation without any risk on your health. Your participation in the study will be completely optional, and all of your personal information will be kept completely secret and used exclusively for the intended purposes of the study. You have the right to withdraw your approval at any moment and without providing a reason even after signing the consent form.

You will be required to sign an informed consent form if you choose to participate in this study

نحن مجموعة من الباحثين في جامعة النجاح الوطنية كلية الطب (نابلس , فلسطين) نريد القيام بدراسة حول مرض الكلى المزمن والعلامات الحيوية لتشخيص المرض , نطلب من حضرتكم مساعدتنا من خلال المشاركة في هذه الدراسة , حيث سيتم سحب عينة دم منك مع العلم انك لن تواجه اي خطر على صحتك ولن يكون هناك مضايقات بمشاركتك سوى وخز الابرة , ستكون مشاركتك هذه اختيارية وطوعية بشكل تام وستكون معلوماتك الشخصية سرية للغاية ولن يتم الاطلاع عليها الا للاشخاص المخولين بذلك ولاهداف الدراسة فقط ,عليك ان تعلم انه يحق لك الانسحاب من المشاركة في اي لحظة ودون ابداء سبب.

يرجى منك التوقيع في حالة موافقتك على المشاركة

توقيع المشارك : ------------

Participant's signature: --------------

Appendix B

Data Collection Sheet

An-Najah National University

Faculty of Graduate Studies

Case report form

Patient registration number: ----- Age: -----

Gender: -----

Patient history of	Yes	Duration
Diabetes		
Hypertension		
Thyroid dysfunction		
Liver disease		
Cardiovascular disease		
Recurrent UTI		
Kidney stone		
Cancer		
Auto immune disease		
Reduction in kidney mass		
Lower urinary tract blocked		

Family history of	Yes	No
Kidney disease		
Kidney stone		
Poly cystic kidney disease		

Patient habits	Yes	No
Taking energy drink		
Constantly taking painkillers		
Drinking enough water		
Making sport		

Patient disease details	Details		
patient initial diagnosis			
Stage of illness			
Way of disease discover	Routine testing	Disease progression	By Chance

	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
Date of visit					
Creatinine result					

Appendix C

Tables

Table 11

Chi-square test

CKD EPI eGFRCr * CKD EPI eGFR cyst c			
	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	284.376[a]	25	.000
CKD EPI eGFRCr * CKD EPI eGFRCr- cyst c			
	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	428.631[a]	25	.000
CKDEPI eGFRCr-cyst c * CKDEPI cyst c			
	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	460.551[a]	25	.000

جامعـــة النجاح الوطنية

كليـــة الدراســـات العليـــا

تطبيق السيستاتين كمؤشر حيوي لتطوير دقة التشخيص عند حالات خلل الكلى المزمن في فلسطين

إعداد

ملاك رائد صالح صالح

إشراف

د. نهاد عثمان

قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في الكيمياء الحيوية السريرية، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس– فلسطين.

2025

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إشراف

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الملخص

المقدمة: ايجاد طريقة مناسبة ودقيقة لقياس معدل الترشيح الكبيبي لتقييم وظيفة الكلى يعد من التحديات المهمة خاصة مع الازدياد الواضح في حالات الاصابة باضطرابات الكلى. حيث أن تقنيات القياس المباشرة التقليدية باهظة الثمن وتستغرق وقتًا طويلاً، فان تقدير معدل الترشيح الكبيبي اصبح التقنية الأكثر استخدامًا وذلك باستخدام مؤشرات داخلية مثل الكرياتينين. وبسبب أن الكرياتينين يتأثر بعوامل خارجية تؤثر على دقته. تشير الابحاث الى ان السيستاتين سي يعد بديلا افضل وادق من الكرياتينين كما ان قيمه ثابتة اكثر بسبب عدم تأثر بالعوامل الخارجية التي تؤثر على الكرياتينين.

الأهداف: هدفت هذه الدراسة إلى اختبار السيستاتين سي كمؤشر حيوي بديل لتقدير معدل الترشيح الكبيبي لدى مرضى الفشل الكلوي المزمن في فلسطين ومقارنة فعاليته بالكرياتينين.

منهجية الدراسة: أجريت هذه الدراسة المقطعية في الفترة ما بين يونيو وديسمبر 2023 على مرضى الفشل الكلوي المزمن من العيادات أو الذين تم قبولهم في مستشفى رام الله الحكومي ومستشفى الوطني الحكومي في نابلس، فلسطين. تم تضمين ما مجموعه 160من المرضى. تم جمع البيانات الديموغرافية والطبية. تم إجراء مستويات الكرياتينين والسيستاتين سي من عينات بلازما المرضى.توظيف نتائج الكرياتينين والسيستاتين سي في معادلات تقدير معدل الترشيح بالكبيبي .

النتائج: تشير نتائج الدراسة إلى أن السيستاتين سي قد يقدم تقديرًا أكثر موثوقية لمعدل الترشيح الكبيبي مقارنة بالكرياتينين، مع استقرار أعلى. كانت المعادلة التي استخدمت كل من الكرياتينين في المصل والسيستاتين سي أفضل من المعادلات التي استخدمت واحدًا فقط من هذه العلامات، حيث قللت من تأثير القيود المستقلة لكلا العلامتين بمفردهما.

الخلاصة: إن تطبيق السيستاتين سي كعلامة حيوية يمكن أن يعزز دقة التشخيص لمرض الكلى المزمن، مما يسمح بالتدخل والإدارة في الوقت المناسب، وبالتالي تحسين نتائج المرضى وتقليل تكاليف الرعاية الصحية المرتبطة بالفشل الكلوي المتقدم. يوصى بإجراء المزيد من البحوث لتعزيز هذه النتائج وتعزيز دمج السيستاتين سي في الممارسة السريرية الروتينية.

الكلمات المفتاحية: مرض الكلى المزمن، معدل الترشيح الكبيبي، السيستاتين سي، الكرياتينين، معادلة وبائيات مرض الكلى المزمن.