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(Research/ Review) Article

The Relationship Between Cystatin-C Levels and The Degree of Anemia in Patients with Stage V Chronic Kidney Disease Undergoing Hemodialysis at RSUD Dr. Mohamad Soewandhie Surabaya

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Abstract: Chronic kidney disease (CKD) is a progressive and irreversible condition with increasing prevalence and a high risk of complications, including anemia in advanced stages. Renal function is often assessed using serum creatinine, but this biomarker is influenced by factors like muscle mass, race, and diet. This highlights the need for more accurate alternative biomarkers. Cystatin C, a sensitive marker of kidney function, is relatively unaffected by muscle mass or race, making it a recommended parameter for estimating glomerular filtration rate (GFR). Despite its potential, studies examining the relationship between cystatin C levels and anemia in CKD patients are limited. This study aimed to investigate the association between cystatin C levels and the degree of anemia in patients with stage V CKD at RSUD dr. Mohamad Soewandhie Surabaya. The observational, cross-sectional study involved 30 patients undergoing hemodialysis. Anemia severity was classified into mild, moderate, and severe categories. The results showed that most patients had moderate anemia (70%), followed by severe anemia (23.3%) and mild anemia (6.7%). The mean cystatin C level was 2.32 ± 1.09 mg/L. Using the Kruskal–Wallis test, no significant differences in cystatin C levels were found across the anemia severity groups ($H = 0.028$; $p = 0.986$). This indicates that cystatin C levels were not significantly associated with the degree of anemia in patients with stage V CKD at the study site. Further research with larger sample sizes is needed to confirm these findings.

Keywords: Cystatin-C; Chronic Kidney Disease; Cross-Sectional Study; Degree of Anemia; Hemodialysis

1. Introduction

Chronic kidney disease (CKD) is one of the leading causes of high morbidity and mortality due to non-communicable diseases (Yu et al., 2025). This disease causes progressive and irreversible kidney damage. Chronic kidney disease (CKD) is defined as an abnormality in kidney structure or function that lasts for at least three months and has an impact on the patient's health (Stevens et al., 2024). Based on the Kidney Disease: Improving Global Outcomes guidelines, the diagnosis of CKD can be confirmed through several criteria, such as albuminuria with an Albumin-to-Creatinine Ratio (ACR) ≥30 mg/g [≥3 mg/mmol], the presence of abnormalities in urine sediment, electrolyte disturbances and other changes due to tubular damage, histological abnormalities in kidney tissue examination, changes in kidney structure detected by imaging, a history of kidney transplantation, and a decreased glomerular filtration rate of less than 60 ml/minute per 1.73 m^2 (KDIGO 2024).

Throughout 2017, the global prevalence of chronic kidney disease (CKD) reached 9.1%. Since 1990, the number of CKD sufferers has increased by 29.3%, with data indicating that approximately 697.5 million people worldwide suffer from chronic kidney disease (CKD). The increase in CKD cases has resulted in a high mortality rate, with 1.2 million people dying in 2017. This mortality rate has increased by 41.5% since 1990, although the age-adjusted increase is only about 2.8%. In 1990, CKD was initially ranked 17th as the leading cause of death, but by 2017, it had risen to 12th, indicating a significant increase in attention (Bikbov et al, 2020).

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Data from the 2023 Indonesian Health Survey indicates that the prevalence of chronic kidney disease (CKD) reached 0.18% of the 277,534,122 population in Indonesia, meaning 638,178 people suffered from chronic kidney disease. The three provinces with the highest incidence rates were Lampung (0.30%), North Sulawesi (0.29%), and West Sulawesi (0.28%). Meanwhile, the prevalence of CKD in East Java reached 0.12%, with 98,738 people diagnosed with CKD.

Chronic kidney disease develops progressively, characterized by a decrease in the glomerular filtration rate until it reaches stage V. At this stage, chronic kidney disease enters the final phase of kidney function decline that requires renal replacement therapy, such as peritoneal hemodialysis, scheduled hemodialysis, or kidney transplantation from a donor (Gusev et al., 2021). At this stage, anemia often appears as a complication, especially in patients undergoing hemodialysis procedures (Stauffer in Daimon, 2024).

The prevalence of anemia increases with decreasing kidney function with an estimated glomerular filtration rate (eGFR) of 30-59 mL/minute/1.73m2(Natale et al., 2022)According to Babitt et al., decreased erythropoietin production, decreased iron absorption through the gastrointestinal tract due to ongoing inflammation, and decreased erythrocyte lifespan are the main mechanisms behind the occurrence of anemia in chronic kidney disease.(Hashmi et al., 2025).

A common method for estimating glomerular filtration rate (GFR) is by measuring serum creatinine levels. Creatinine is a byproduct of creatine phosphate metabolism in skeletal muscle, and its levels are influenced by muscle metabolism and dietary creatine intake, including meat and creatine supplements.(Benoit, Ciccia, and Devarajan, 2020)Serum creatinine levels are highly dependent on muscle mass, so in patients with low muscle mass, creatinine-based eGFR tends to overestimate the true GFR, while in patients with high muscle mass, the value may be lower. Furthermore, consuming cooked meat can also increase blood creatinine levels.(Groothof et al., 2022)Therefore, an alternative method that is more stable and accurate is needed.

*Cystatin-C*This is a new marker of kidney function that is now being introduced. This substance is continuously produced by cells in the body and is not secreted in the renal tubules.(Arifin and Kurniawan, 2016). According to Esezobor et al., in the studyMohammed et al., (2023)Cystatin-C is a small protein with a molecular weight of 13.3 kDa from the cystatin family that acts as a cysteine protease inhibitor. This protein is constantly produced by all nucleated cells.The American Society of Nephrology and National Kidney Foundation (2021) guidelines recommend wider use of cystatin-C because it is not influenced by race and when combined with creatinine, can estimate GFR more accurately.(Delgado et al., 2022). Based on Kidney Disease: Improving Global Outcomes (KDIGO, 2012), it is recommended to measure cystatin-C in adults with eGFR ranging from 45-60 mL/minute/1.73 m², especially in situations where there are no other indicators to show kidney damage, to ensure the accuracy of the diagnosis of chronic kidney disease.(Visinescu et al., 2024).

According to Grubb A et al., initial research on cystatin-C as a marker of GFR indicates that increased plasma levels not only reflect decreased kidney function, but also play a role in significant changes in the protein profile in the body of patients with kidney disease.(Malmgren et al., 2023).

Arifin and Kurniawan's research showed that serum cystatin-C has a higher sensitivity of 82.4%, compared to serum creatinine, which only reached 52.9% in detecting decreased kidney function in patients with chronic kidney disease (CKD). However, both markers have the same specificity of 85.7%. However, to date, there is still a limited number of studies specifically comparing the use of cystatin-C levels in assessing the degree of anemia in CKD patients at Dr. Mohamad Soewandhie General Hospital, Surabaya. Therefore, this study aims to examine the relationship between cystatin-C levels and the degree of anemia in CKD patients at the hospital.

2. Literature Review

Kidney Organ

A. Kidney Anatomy

The kidneys are normally paired and located in the retroperitoneal region. In humans, they are located between the twelfth thoracic vertebra and the third lumbar vertebra, on opposite sides of the vertebral column. The right kidney is generally located slightly lower toward the back of the body than the left. In adult men, each kidney weighs approximately 125 to 170 grams, while in adult women, it weighs approximately 115 to 155 grams. The kidneys generally

measure approximately 11 to 12 cm in length, 5.0 to 7.5 cm in width, and 2.5 to 3.0 cm in thickness.(Brenner & Rector's The Kidney, 2019).

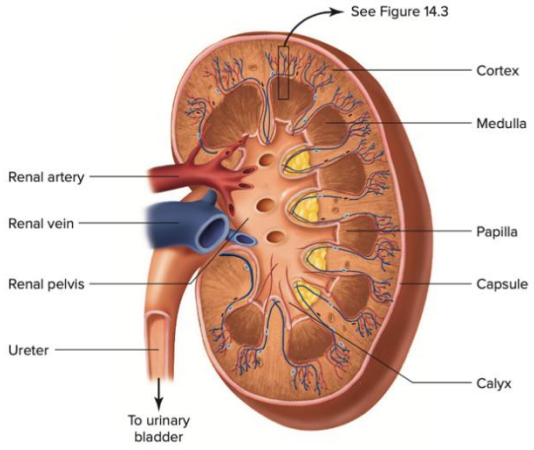

Figure 1. Kidney Structure
Source: Vander's, 2018.

The kidney has a medial hilum, which serves as the entry and exit point for blood vessels, nerves, lymphatic vessels, and the ureter, which functions to drain urine into the urinary bladder. Anatomically, the kidney is surrounded by a fibrous capsule and composed of the cortex and medulla, which form the renal pyramid, which empties into the renal papilla, calyces, and renal pelvis, the initial part of the ureter. The walls of the calyces, renal pelvis, and ureter are composed of smooth muscle that contracts rhythmically to propel urine toward the bladder before being excreted from the body (Guyton and Hall, 2020).

The glomerulus and renal tubule form the functional unit of the kidney called the nephron, which numbers approximately one million in humans. At the end of the nephron is the Bowman's capsule, which contains the glomerulus, the site of blood filtration, where blood enters through the afferent arteriole and exits through the efferent arteriole. The filtration process is supported by a filtration membrane composed of capillary endothelium, basement membrane, and podocyte cells, and is controlled by mesangial cells that play a role in regulating glomerular filtration (Barrett et al., 2019). The filtered fluid flows from the Bowman's capsule to the proximal tubule, loop of Henle, and distal tubule, with regulation of kidney function and blood pressure involving the juxtaglomerular apparatus (Sherwood, 2019).

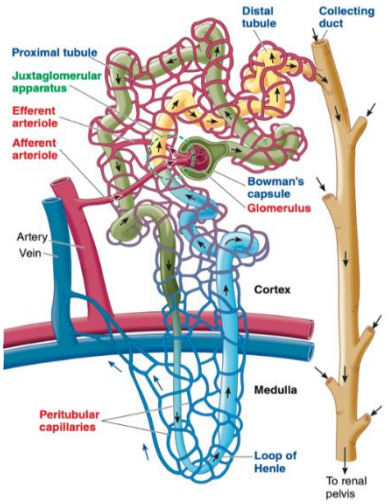

Figure 2. Nephron Structure
Source: Sherwood, 2019.

B. Kidney Function

The kidneys play a crucial role in maintaining bodily balance by regulating electrolyte volume, acid-base balance, blood volume, blood pressure, and maintaining stable extracellular fluid (ECF) volume. They maintain electrolyte balance despite changes in intake and output, and regulate urine volume to compensate for fluid loss due to excessive sweating, vomiting, diarrhea, or bleeding (Sherwood, 2019). They excrete metabolic waste products, toxins, and drugs, regulate water and electrolyte balance, control body fluid osmolality, regulate arterial pressure, maintain acid-base balance, produce erythropoietin, secrete and metabolize hormones, and produce glucose through gluconeogenesis during prolonged fasting (Guyton and Hall, 2020).

C. Kidney Physiology

The kidneys play a role in filtering blood plasma and regulating the excretion of various substances from the filtrate according to the body's physiological needs. Unnecessary substances are excreted through urine, while useful compounds are reabsorbed into the blood (Guyton & Hall, 2020). Urine formation involves three main processes: glomerular filtration, reabsorption, and tubular secretion. In glomerular filtration, fluid flows through three filtering layers: the capillary endothelium, the basement membrane, and the inner layer of Bowman's capsule, which prevents blood cells and plasma proteins from entering the filtrate (Sherwood, 2019). Approximately 20% of plasma is filtered at a filtration rate of approximately 125 mL per minute, or 180 liters per day, followed by reabsorption of useful substances and secretion of certain substances, resulting in urine being excreted from the body (Sherwood, 2019).

D. Chronic Kidney Disease

Chronic Kidney Disease (CKD) is a condition of impaired kidney function characterized by kidney damage or a decrease in the estimated glomerular filtration rate (eGFR) below 60 mL/minute/1.73 m^2 that lasts at least three months (Vaidya & Aeddula, 2024). The diagnosis of CKD according to the 2024 Kidney Disease: Improving Global Outcomes (KDIGO) guidelines is determined based on decreased eGFR, increased urinary albumin-to-creatinine ratio, urinary sediment abnormalities, electrolyte disturbances due to tubular damage, histopathological evidence, structural changes in the kidney, or a history of kidney transplantation (KDIGO, 2024).

The classification of CKD is based on etiology, glomerular filtration rate (G1–G5), and albuminuria level (A1–A3), with the main emphasis on GFR and albuminuria parameters as determinants of disease severity and risk of complications (KDIGO, 2024).

Chronic kidney disease can be caused by diabetes mellitus, hypertension, primary and secondary glomerulonephritis, chronic tubulointerstitial nephritis, genetic or cystic kidney disease, vasculitis, plasma cell disorders, and sickle cell nephropathy (Vaidya and Aeddula, 2024). Risk factors for CKD are divided into modifiable factors such as hypertension, proteinuria, activation of the renin-angiotensin-aldosterone system, obesity, smoking, dyslipidemia, and hyperuricemia, as well as non-modifiable factors such as age, gender, ethnicity, and genetic factors (Vaidya and Aeddula, 2024).

Chronic kidney disease (CKD) results from a decline in kidney function, characterized by a decreased glomerular filtration rate and increased albuminuria. The pathophysiology of CKD involves specific mechanisms such as genetic disorders, glomerular inflammation, and toxic exposure, as well as nonspecific mechanisms such as hyperfiltration and hypertrophy of the remaining nephrons due to the influence of vasoactive hormones, cytokines, and growth factors. These adaptations are maladaptive and lead to damage to the filtration barrier, glomerular sclerosis, and progressive nephron loss (Bargman and Skorecki, 2022).

E. Structure and Function of the Nephron

The kidneys are composed of functional units called nephrons, which in humans number approximately one million and play a key role in blood filtration. Each nephron consists of a glomerulus and a tubular system. The glomerulus is located within Bowman's capsule and is composed of a network of capillaries that receive blood from the afferent arteriole and channel it through the efferent arteriole, with differences in diameter that support filtration pressure. The blood filtration process occurs through a filter layer consisting of the capillary endothelium, the glomerular basement membrane, and podocytes that form the filtration slit. Furthermore, mesangial cells play a role in regulating the filtration rate and contribute to the pathogenesis of glomerular disease. The formed filtrate then flows through the proximal tubule, the loop of Henle, and the distal tubule before returning to the glomerulus, forming the juxtaglomerular apparatus, which plays a crucial role in regulating blood pressure and balancing renal filtration (Barrett et al., 2019; Sherwood, 2019).

Chronic kidney disease (CKD) is a global health problem, with a prevalence of 9.1% in 2017 and an estimated 697.5 million people worldwide. Since 1990, the number of CKD cases

has increased significantly along with increasing life expectancy and the increasing prevalence of risk factors such as diabetes and hypertension. This increase has resulted in high mortality rates, with CKD rising to become the leading cause of death globally. In Indonesia, the prevalence of CKD in 2023 was recorded at 0.18%, with the highest rates found in the provinces of Lampung, North Sulawesi, and West Sulawesi. The prevalence of CKD also increases with age, with the highest proportion in the elderly, particularly those aged ≥75 years (Bikbov et al., 2020; KDIGO, 2024; SKI, 2023).

A diagnosis of CKD is made when there are abnormalities in kidney structure or function that persist for at least three months. Kidney damage can be demonstrated by an increased urinary albumin-to-creatinine ratio, urinary sediment abnormalities, persistent hematuria, electrolyte disturbances due to tubular dysfunction, histological findings, radiological imaging, or a history of kidney transplantation. Additionally, a decrease in the glomerular filtration rate below 60 mL/min/1.73 m^2, which includes categories G3a to G5, is also a basis for diagnosis. The persistent presence of any one of these criteria for more than three months is sufficient to confirm a diagnosis of CKD (KDIGO, 2024).

The clinical manifestations of CKD are highly variable and require a thorough physical examination to identify the underlying cause. Evaluation of volume status is crucial because patients may experience volume depletion due to poor oral intake or volume overload due to conditions such as heart failure, liver failure, or nephrotic syndrome. A history of hypertension and chronic diabetes may be indicated by retinal changes, while a vascular bruit may suggest renovascular disease. Flank pain or kidney enlargement may indicate obstructive uropathy or certain kidney diseases. CKD may also be accompanied by neurological and dermatological manifestations, such as neuropathy, skin rashes, purpura, telangiectasias, and sclerosis, depending on the underlying systemic disease (Kyneissia Gliselda, 2021).

F. Hemodialysis

Hemodialysis is a renal replacement therapy that aims to remove metabolic waste products and toxic substances from the blood and improve fluid and electrolyte balance due to decreased kidney function. This procedure uses a semipermeable membrane to filter blood outside the body, mimicking the function of nephrons. In addition to improving survival, hemodialysis also impacts patients' quality of life, requiring long-term physical and psychological adjustments (Saputra and Wiryansyah, 2023; Agus Tiar et al., 2022).

Patients with advanced CKD are encouraged to receive early education regarding renal replacement therapy options. Dialysis access evaluation and preparation are recommended for patients with a significant decrease in eGFR, particularly in the range of 15–20 mL/minute/1.73 m^2, or if there is a rapid and unstable decrease in eGFR. This approach aims to ensure timely initiation of renal replacement therapy according to the patient's clinical condition (Ministry of Health, 2023).

Hemodialysis works based on the principles of diffusion and convection through a semipermeable membrane due to the concentration difference between blood and dialysate. Blood is transported into the dialyzer with the help of an integrated machine system, allowing for the removal of metabolic waste, electrolyte regulation, and acid-base balance correction. The most recommended vascular access is an arteriovenous fistula due to its better long-term durability, although it takes time to reach functional maturity. A temporary alternative, a dialysis catheter, can be used if definitive access is not yet available, but carries a higher risk of complications (Brewster and Turner, 2017; Dirkx and Woodell, 2025).

Hemodialysis is generally safe, but it can be accompanied by acute complications such as hypotension, muscle cramps, nausea, vomiting, dialyzer reactions, and cardiovascular disorders. Hypotension is the most common complication and is associated with excessive ultrafiltration, autonomic disorders, and low cardiac reserve. Dialyzer reactions can be allergic or non-allergic, with varying clinical manifestations. Prevention and management of complications require appropriate ultrafiltration settings, dry weight assessment, and close monitoring during the dialysis procedure (Liu and Chertow, 2022; Raja and Seyoum, 2020).

G. Anemia

Anemia is a condition in which hemoglobin levels in men are below 13 g/dL and in women below 12 g/dL. In patients with chronic kidney disease (CKD), anemia is a common complication and is generally normocytic, normochromic, and hypoproliferative. The presence of anemia in CKD patients can worsen clinical conditions, reduce quality of life, and increase the risk of death (Hashmi et al., 2025). Anemia is most often detected through low hemoglobin (Hb) levels and remains the main approach in clinical practice and public health monitoring (Chaparro and Suchdev, 2019).

Anemia is functionally classified into hypoproliferative anemia, anemia due to impaired red blood cell maturation, and anemia due to increased red blood cell destruction or loss. In

hypoproliferative anemia, reticulocyte counts are low, and red blood cell morphology is generally normocytic, normochromic. Hemolytic anemia is characterized by significantly increased reticulocyte production, while anemia due to bleeding shows a more limited erythropoietic response (Bargman and Skorecki, 2022). Based on hemoglobin levels, anemia is divided into mild, moderate, and severe anemia (Helmyati et al., 2023).

Anemia in CKD patients is usually caused by inadequate erythropoietin production. Other factors contributing to anemia include iron deficiency, shortened erythrocyte lifespan, secondary hyperparathyroidism, and chronic infection and inflammation (Ministry of Health of the Republic of Indonesia, 2023). Decreased erythropoietin production due to impaired kidney function is the primary cause of anemia in CKD, along with other factors such as micronutrient deficiencies, malnutrition, blood loss, inflammation, and the use of certain medications (Purnamasari, 2023). In stage 3 CKD, when the glomerular filtration rate decreases below 60 mL/minute/1.73 m^2, erythropoietin production is generally insufficient, resulting in normochromic, normocytic anemia (Provenzano, 2017).

According to 2019 global data, the prevalence of anemia reached 29.9%, with a higher prevalence in Southeast Asia at 41.9%. In Indonesia, the prevalence of anemia among women of reproductive age reached 30.6%, with an increase in adolescent girls from 22.7% to 32% (Rahman and Fajar, 2024). In CKD patients undergoing hemodialysis, normochromic normocytic anemia is the most common type (Aisyafitri, Uwan, and Fitriangga, 2018).

Dysregulation of erythropoietin (EPO) is the primary mechanism for anemia in CKD. EPO is a glycoprotein hormone produced by renal interstitial cells in response to hypoxia and plays a crucial role in erythropoiesis. In CKD patients, kidney tissue damage leads to reduced EPO production despite low hemoglobin levels (Badura et al., 2024). Furthermore, impaired iron regulation and chronic inflammation increase hepcidin levels, which inhibits iron absorption and release. This condition leads to functional iron deficiency and contributes to refractory anemia in CKD patients (Badura et al., 2024). Other factors such as medication use and bleeding also exacerbate anemia.

The diagnosis of anemia in CKD patients is based on hemoglobin levels, transferrin saturation (TSAT), and serum ferritin. Absolute iron deficiency is characterized by a TSAT <20% and low ferritin, while functional iron deficiency is characterized by a TSAT <20% with elevated ferritin (Babitt et al., 2021). Anemia screening is recommended in all patients with CKD stage 3 or higher and includes evaluation of hemoglobin, ferritin, and TSAT. Additional tests such as C-reactive protein (CRP), vitamin B12, and folate are needed to avoid misinterpretation due to inflammation and to rule out other causes of anemia (Hain et al., 2023; Badura et al., 2024).

H. Erythropoiesis and the Role of Erythropoietin

Erythropoiesis is the continuous process of red blood cell formation in the bone marrow to maintain erythrocyte balance and ensure adequate oxygen transport to tissues. Decreased blood oxygen levels stimulate the kidneys to produce the hormone erythropoietin (EPO), which plays a key role in the final phase of erythropoiesis by stimulating the proliferation and differentiation of erythrocyte precursors. Erythrocyte production increases significantly in hypoxic conditions, such as anemia, heart and lung disease, strenuous physical activity, and exposure to high altitudes. The erythron concept describes the functional unity between mature erythrocytes, bone marrow precursors, and progenitor cells in understanding the physiology and pathology of erythropoiesis (Sherwood & Ward, 2019; Widmaier et al., 2019; Wintrobe's, 2022).

Iron is an essential component in hemoglobin formation and erythropoiesis, with transferrin serving as the primary iron transport protein in plasma. In iron deficiency anemia, iron stores in macrophages and plasma iron levels are decreased, inhibiting erythrocyte formation and resulting in microcytic, hypochromic red blood cells. Conversely, in inflammatory anemia, iron stores are relatively normal or increased, but iron release into the circulation is inhibited by the inflammatory process, reducing iron supply to the bone marrow. Morphologically, inflammatory anemia is generally characterized by normocytic, normochromic red blood cells (Robert J. Means et al., 2022).

I. Kidney Function Biomarker: Creatinine

Kidney function is commonly assessed by estimating the glomerular filtration rate (eGFR) using serum creatinine as an endogenous marker. Creatinine is a metabolic byproduct of muscle creatine, which is largely eliminated through glomerular filtration, with varying contributions from tubular secretion. The creatinine-based MDRD and CKD-EPI equations are widely used to estimate eGFR, but creatinine levels are significantly influenced by muscle mass, meat intake, age, and certain clinical conditions. These limitations make creatinine less sensitive in detecting early kidney disease and potentially bias estimates in individuals with extreme muscle mass, although it remains the primary method in clinical practice (Stehlé and Delanaye, 2024; Groothof et al., 2022; Spencer, Desborough, and Bhandari, 2023).

*Cystatin-C*Cystatin-C is a small protein constantly produced by all nucleated cells and freely filtered by the glomerulus, making it a more stable alternative biomarker for assessing kidney function. Unlike creatinine, cystatin-C levels are relatively unaffected by muscle mass, gender, or body weight, although they can still be influenced by non-renal factors such as inflammation, thyroid disorders, obesity, and corticosteroid use. The combination of cystatin-C and creatinine-based eGFR has been shown to improve the accuracy of GFR estimation and support clinical decision-making, including the prediction of cardiovascular risk and mortality. Therefore, cystatin-C is recommended as a complementary or alternative biomarker in the evaluation of kidney function (Markos et al., 2020; Gottlieb et al., 2023; Ding, Liu, and Wang, 2022).

Cystatin-C-based eGFR formulas, specifically the CKD-EPI cystatin-C, are considered superior to creatinine-based formulas due to their minimal influence on demographic factors. This formula provides a more accurate estimate of kidney function, improves the accuracy of clinical risk stratification, and is better at predicting cardiovascular events and mortality, potentially becoming an important standard for assessing kidney function in the future (Cusumano, Tzanno-Martins, and Rosa-Diez, 2021; Zou et al., 2020).
The following is the 2012 CKD-EPI equation based on cystatin-C:

$$\text{Estimated Glomerular Filtration Rate (mL/min/1.73 m}^2) =$$
$$= 133 \times \min(\text{Scys}/0.8, 1)^{-0.499} \times \max(\text{Scys}/0.8, 1)^{-1.328} \times 0.996^{\text{Age}} [\times 0.932 \text{ if female}]$$

Figure 2 Cystatin-C based CKD-EPI 2012 equation
Source:Cusumano, Tzanno-Martins and Rosa-Diez, 2021.

Information:
eLFG: estimated glomerular filtration rate (mL/min/1.73 m²)
Scys: standardized serum cystatin-C level (mg/L)
min: minimum value between Scys/0.8 or 1
max: maximum value between Scys/0.8 or 1
Age: age (years)

J. Cystatin-C Laboratory Examination Method with ELISA

Enzyme-linked immunosorbent assay(ELISA) is a widely used immunological method for detecting and quantifying proteins, including cystatin-C, based on the specific binding of an antigen to an antibody labeled with an enzyme. The reaction between the enzyme and the substrate produces a signal in the form of a color change or fluorescence that can be measured qualitatively or quantitatively. In conventional ELISA, chromogenic or fluorogenic substrates such as TMB, ABTS, and OPD are used as the detection signal source. However, the sensitivity of this method is relatively limited due to the low extinction coefficient of small molecule substrates. The development of ELISA using plasmonic nanoparticles, particularly gold, can significantly increase sensitivity due to its much higher extinction coefficient through the localized surface plasmon resonance (LSPR) phenomenon, thus enabling more accurate detection of cystatin-C at low concentrations (Dita, 2021; Gao et al., 2020).

K. The Relationship Between Cystatin-C Levels and Anemia

Several previous studies have shown a link between cystatin-C levels, kidney function, and anemia. Cystatin-C has been shown to be a more sensitive biomarker than creatinine in detecting impaired kidney function at an early stage and is associated with morbidity, mortality, and progression of chronic kidney disease. Impaired kidney function contributes to anemia, primarily through decreased production of erythropoietin, which plays a crucial role in erythropoiesis. Epidemiological studies have shown that in individuals with anemia, cystatin-C-based kidney function estimation results in a higher prevalence of kidney disease than creatinine-based methods, particularly in younger age groups, women, and individuals

with systemic inflammation. This indicates that cystatin-C can more accurately reflect kidney function and detect kidney disorders earlier in anemic populations. Thus, these findings support a significant association between cystatin-C levels and anemia and strengthen the theoretical basis of this study (Estrella et al., 2010; Benoit, Ciccia, and Devarajan, 2020; Bila et al., 2025).

3. Materials and Method

This study is a preliminary observational analytical study using a cross-sectional method that aims to evaluate the relationship between cystatin-C levels and the degree of anemia in patients with stage V chronic kidney disease (CKD) undergoing hemodialysis. The study was conducted in August–September 2025 at Dr. Mohamad Soewandhie General Hospital, Surabaya, and the Ciputra University Laboratory, Surabaya. The study population was all stage V CKD patients undergoing hemodialysis at Dr. Mohamad Soewandhie General Hospital, Surabaya, with a sample of 30 subjects selected using a total sampling technique based on predetermined inclusion and exclusion criteria. The research instruments included examination of cystatin-C levels using the ELISA method from blood serum samples and data on the degree of anemia obtained from patient medical records. Data collection techniques were carried out through a single blood draw by laboratory staff and medical record data searches. The data analysis technique was carried out bivariately using the Kruskal–Wallis test because the degree of anemia is ordinal data and cystatin-C levels are numeric data that are not normally distributed, with data processing using the IBM Statistical Package for the Social Sciences (SPSS) application and a significance level of 0.05.

4. Results and Discussion

Research Overview

The type of research used is an analytical observational with a cross-sectional design that provides an examination of the subjects studied. The study was conducted by examining cystatin-C levels in patients who met the inclusion and exclusion criteria. Examination of cystatin-C levels used the Enzyme-Linked Immunosorbent Assay (ELISA) method as one of the blood serum-based laboratory analysis methods. The population distribution was taken in August–September 2025 at Dr. Mohamad Soewandhie Hospital Surabaya in patients with stage V Chronic Kidney Disease undergoing hemodialysis. Sampling was carried out using the total sampling method, with a total of 30 patients who underwent cystatin-C level examinations and medical record data collection to determine the degree of anemia.

Respondent Characteristics

Respondent characteristics in this study included baseline patient data such as gender, age, and degree of anemia. The results of the respondent data collection are presented below:

Table 1. Respondent Characteristics

C		n	%	Mean ± SD	Median (Min-Max)
Gender	Man	20	66.7		
	Woman	10	33.3		
Age	All	30	100	51.33 ± 8.74	52.50 (34–64)
	30-39	4	13.3		
	40-49	6	20.0		
	50-59	16	53.3		
	≥ 60	4	13.3		
Body Weight (kg)				61.5 ± 12.07	59.5 (36–88)
Hemoglobin (g/dL)				8.78 ± 1.17	8.50 (6.3–11.2)
Cystatin-C				2.32 ± 1.09	2.30 (0.61–4.83)
Degree of Anemia	Light	2	6.7	2.29 ± 0.39	2.29 (2.02–2.57)
	Currently	21	70.0	2.31 ± 1.23	2.23 (0.61–4.83)
	Heavy	7	23.3	2.35 ± 0.81	2.36 (1.06–3.69)

Based on Table 1, this study involved 30 patients with stage V chronic kidney disease (CKD) undergoing hemodialysis at Dr. Mohamad Soewandhie Regional General Hospital, Surabaya. Respondents consisted of 20 men (66.7%) and 10 women (33.3%). The average age of respondents was 51.33 ± 8.74 years, with a median of 52.50 years and an age range of 34

to 64 years. The distribution of age groups shows that the majority were in the 50–59 years group, with 16 people (53.3%), followed by the 40–49 years group with 6 people (20.0%), and the 30–39 years and ≥60 years groups, each with 4 people (13.3%).

Patient weight ranged from 36 to 88 kg, with a mean of 61.5 ± 12.07 kg and a median of 59.5 kg, indicating a variation in anthropometric status in this study population. Hemoglobin values ranged from 6.3 to 11.2 g/dL, with a mean of 8.78 ± 1.17 g/dL and a median of 8.50 g/dL, indicating that most patients were anemic. Cystatin-C levels in respondents ranged from 0.61 to 4.83 mg/L, with a mean of 2.32 ± 1.09 mg/L and a median of 2.30 mg/L, reflecting variations in the degree of kidney dysfunction in stage V CKD patients. When viewed based on the degree of anemia, 21 people (70%) had moderate anemia, 7 people (23.3%) had severe anemia, and 2 people (6.7%) were classified as mild anemia.

Bivariate Test

The bivariate test in this study was conducted to analyze the relationship between cystatin-C levels and the degree of anemia in patients with stage V chronic kidney disease. The analysis used the Kruskal-Wallis test because the cystatin-C level variable is on a ratio scale and the degree of anemia is on an ordinal scale. The results of the bivariate analysis are presented in the following table:

Table 2. The Relationship between Cystatin-C Levels and the Degree of Anemia in Patients PGK Stage V at Dr. Mohamad Soewandhie Regional Hospital, Surabaya 2025.

Variables	Category	n	Median (Min-Max)	*p-value*
Cystatin-C Level (mg/L)	Mild Anemia	2	2.29 (2.02–2.57)	0.986
	Moderate Anemia	21	2.23 (0.61–4.83)	
	Severe Anemia	7	2.36 (1.06–3.69)	

Kruskal-Wallis test

Based on the tabulation results in table 2 above, the majority of respondents experienced moderate anemia, namely 21 people. Seven respondents had severe anemia, while two respondents had mild anemia.

The distribution of anemia degrees is also shown through the median, minimum, and maximum values for each category. For mild anemia, the median was 2.29 with a range of 2.02–2.57. For moderate anemia, the median was 2.23 with a range of 0.61–4.83. For severe anemia, the median was 2.36 with a range of 1.06–3.69.

Analysis of the relationship between cystatin-C levels and anemia severity was performed using the Kruskal-Wallis test. The Kruskal-Wallis test results showed no significant difference between cystatin-C levels in the mild, moderate, and severe anemia groups ($H = 0.028$; $p = 0.986$). Furthermore, a p value > 0.05 indicates that the relationship is not statistically significant. Thus, no significant relationship was found between cystatin-C levels and anemia severity in patients with stage V chronic kidney disease.

Discussion

This study included 30 patients with stage V CKD undergoing hemodialysis, where blood samples were taken prior to hemodialysis. Respondent characteristics indicated that most patients were in the 50–59 year age group and were predominantly male. Variations in age and gender in this study population did not affect the interpretation of cystatin-C levels, as this biomarker is not influenced by non-renal factors such as age, gender, or race. This is consistent with the explanation that the cystatin-C-based kidney function estimation formula has better accuracy than creatinine because it is not affected by demographic differences. Therefore, cystatin-C levels in this study can more objectively reflect kidney filtration function (Cusumano, Tzanno-Martins, & Rosa-Diez, 2021). Therefore, cystatin-C levels in this study can more objectively reflect kidney filtration function.

Cystatin-C levels in patients showed a fairly wide range, namely 0.61–4.83 mg/L, with a mean of 2.32 ± 1.09 mg/L. This variation indicates differences in the level of glomerular filtration impairment in each patient. This finding is consistent with research findings that show that cystatin-C levels are increased in CKD patients compared to the healthy population, suggesting that this biomarker can be used as a marker of kidney dysfunction.(Noman Salman, Jasim kzar, and Hamzah, 2022)This agreement indicates that the pattern of cystatin-C levels in this study is still in accordance with the existing literature.

The majority of patients in this study had moderate anemia (70%), followed by severe anemia (23.3%) and mild anemia (6.7%). Although anemia is very common in CKD, the Kruskal-Wallis test showed no significant difference between cystatin-C levels in the mild, moderate, and severe anemia groups ($H = 0.028$; $p = 0.986$). This indicates that changes in cystatin-C levels do not correlate with anemia severity in the CKD stage V population.

This lack of association may be due to the multifactorial pathophysiological mechanisms of anemia in CKD, which are independent of glomerular filtration biomarkers. The primary cause of anemia in CKD is decreased erythropoietin (EPO) production due to damage to type I interstitial cells in the renal cortex. Studies have shown that structural kidney damage reduces the capacity of EPO-producing cells, resulting in decreased erythropoiesis, although the degree of renal function decline can vary between patients (Badura et al., 2024). This mechanism aligns with research by Bargman and Skorecki, which confirmed that decreased EPO production due to kidney tissue damage is the primary cause of normocytic, normochromic anemia in CKD (Bargman & Skorecki, 2022). Therefore, although cystatin-C increases with decreased filtration function, this biomarker is not directly related to the erythropoiesis process.

In addition to EPO deficiency, chronic inflammation plays a key role in worsening anemia in CKD. Increased proinflammatory cytokines such as IL-6 and TNF-α stimulate the production of hepcidin, a hormone that inhibits intestinal iron absorption and release from macrophages. This condition results in functional iron deficiency despite high ferritin stores (Badura et al., 2024). Because this iron regulation is not related to glomerular filtration, cystatin-C levels do not correlate with the severity of anemia.

A study by Purnamasari (2023) found that other multifactorial factors such as blood loss due to hemodialysis procedures, gastrointestinal bleeding, malnutrition, vitamin B12 and folate deficiencies, aluminum toxicity, use of ACE inhibitors or ARBs, and impaired platelet function also exacerbate anemia. Provenzano (2017) emphasized that anemia in CKD is influenced by the interaction between inflammation, nutritional disorders, EPO resistance, and chronic blood loss. Because these factors are not related to cystatin-C, this biomarker cannot be used to predict anemia severity.

The results of this study are consistent with existing physiological understanding, as cystatin-C reflects impaired glomerular filtration, while anemia in CKD is primarily caused by decreased erythropoietin production, chronic inflammation, impaired iron metabolism, and nutritional and bleeding factors. This difference in underlying mechanisms explains the lack of a relationship between cystatin-C levels and anemia severity in stage V CKD patients in this study.

5. Conclusion

The measurement results showed that cystatin-C levels in stage V CKD patients at Dr. Mohamad Soewandhie Regional General Hospital, Surabaya, varied between research subjects based on ELISA examination. Measurement of hemoglobin levels shows variations in the severity of anemia in stage V CKD patients, which are classified into mild, moderate, and severe anemia. Statistically, no significant relationship was found between cystatin-C levels and the degree of anemia in stage V CKD patients ($H = 0.028$; $p = 0.986$).

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