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Evaluation of Homocysteine, D-dimer and Biochemical Parameters in Relation to Chronic Kidney Disease Stages among Patients Attending Specialist Hospitals in Enugu, Enugu State.

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1. Introduction

1.1 Background of the study

Chronic kidney disease (CKD), a life threatening heterogeneous disorder, has become one of the fastest growing global public health problem associated with increased morbidity and mortality [1]. This progressive disease condition is characterized by an irreversible gradual structural and functional loss of the kidney over 3 months or years showing declined estimated glomerular filteration rate (eGFR) (<60ml/min/1.73m^2) and albuminuria [2]. Current Chronic studies stated that chronic kidney disease ranked the 6th fastest growing cause of death worldwide with >10% of the general population amounting 850 million being affected and at least 2.4 million deaths recorded annually [3]. Apparently, this alarming prevalence could be attributed to the clinically silent onset of chronic kidney disease with few non-specific symptoms until later stages usually with adverse outcomes and also to the increasing presence of its comorbidities in the general population. Despite its high prevalence and associated complications, CKD chronic kidney disease awareness remains profoundly low.

Though the elderly is mostly affected, it affects all age groups diminishing the quality of life with profound socioeconomic consequences in both developed and developing countries. Previous studies have shown that hypertension, diabetics, glomerulonephritis, cardiovascular disease and advanced age are the major causes and comorbidities of chronic kidney disease [4]. Kidney disease improving global outcome (KDIGO) 2022 guideline, classified chronic kidney disease into five stages based on eGFR and albuminuria status to aid accurate assessment of disease severity with informed management decisions thereby averting progression to end stage renal disease (ESRD) where treatment could only be with dialysis or kidney transplantation [5]. Currently, chronic kidney disease is not curable and its management relies on treatments that prevent chronic kidney disease progression and cardiovascular disease.

Electrolytes, urea and creatinine panel are key factors in determining the efficacy of kidney functions. They provide direct evidence of kidneys excretory function, regulation of extracellular fluid volume and maintenance of acid base balance. Previous studies have documented electrolyte imbalance as one of the leading complications of chronic kidney disease with increased mortality rate mostly in end state renal disease [6]. The attributed electrolyte disorders in renal function deterioration including sodium, potassium, magnesium, bicarbonate, chloride and phosphorus are known to cause serious complications like protein energy wasting, vascular calcification, bone demineralization and even death [7]. Sodium is an important extracellular cation responsible for maintenance of fluid balance and plasma volume with its imbalance resulting to fluid overload (edema), changes in blood pressure, delirium, seizure and eventually death [8]. Potassium levels is frequently implicated in chronic kidney disease as its elevated levels could be critical and life threatening in the management of chronic kidney disease. Potassium functions in the maintenance of membrane resting potentials, neurotransmitter release and muscle contraction [9]. Reports have shown that elevated serum potassium levels predisposes individuals to greater risk of end stage renal disease, arrhythmias, sudden cardiac arrest and mortality [10].

Metabolic acidosis reported in chronic kidney disease is the acid build up that occurs due to impaired kidneys' ability to excrete acid and regenerate bicarbonate. Previous studies have documented a direct correlation between declined glomerular filtration rates with reduced bicarbonate overly caused by decreased ammonia production, impaired regeneration of bicarbonate and reduced hydrogen ion excretion [11]. Urea and creatinine are the vital factors in renal function deterioration and the cornerstone of chronic kidney disease diagnosis and staging [12]. While urea is the metabolized product of dietary proteins, creatinine is produced from muscle with the both being excreted through the kidneys as metabolic waste. Their accumulation due to renal function impairment is toxic and detrimental to human health. Haemodialysis is the replacement therapy that plays an important role in the extracorporeal removal of waste products including urea, creatinine and free water from the blood [10].

Cardiovascular disease (CVD) are the leading cause of mortality in chronic kidney disease patients. Growing evidence has shown that cardiovascular is responsible for about 40% of hospitalization and 50% of mortality in chronic kidney disease patients at all stages and in end stage renal disease; CVD mortality rate is 10-20 times higher than the general population [13]. Though CKD progression may lead to renal insufficiency, patients may likely die from cardiovascular related complications before reaching end stage renal disease. In chronic kidney disease patients, there is increased reactive oxygen species (ROS) production [14]. The outcome of excess reactive oxygen species allows the existence of free radicals which

circulates the body systems. It has been postulated that the imbalance between endogenous mitochondrial reactive oxygen species production and removal due to its overproduction or depletion of antioxidant defense activity gives rise to oxidative stress [15]. Studies have shown possible correlation between oxidative stress and increased progression rate of kidney damage [16, 17]. These free radicals worsen kidney failure by inducing apoptosis and increasing inflammation and fibrosis of the renal tissue.

Homocysteine (HCy), a sulfur containing amino acid generated from methionine metabolism in the kidney have been implicated by recent studies as a biochemical marker in the development and progression of renal disease [18]. The existing relationship between homocysteine and kidney function is bidirectional in that accumulation of homocysteine damages the glomerular cells leading to accelerated renal function impairment which consequently, further increases homocysteine levels. Therefore, high levels of homocysteine is an independent risk factor for chronic kidney disease [19]. Hyperhomocysteinemia which is reported in greater percentage in chronic kidney disease patients especially in end stage renal disease is linked to impaired renal metabolism and reduced renal excretion of homocysteine. This occurs possibly due to Vitamin B6, B12 and folate deficiency, genetic defect and other pathophysiological conditions driven by oxidative stress [20]

There is ample evidence that homocysteine causes endothelial cell damage, increased coagulation factor activities and impaired fibrinolytic potentials. Also, it promotes proliferation of smooth muscle cells resulting to several interactions with platelets, clotting factors and lipids hence its high levels association with thrombosis [21, 22]. Homocysteine attracts attention not just for its close relationship with renal function but has also been implicated by previous studies as an independent risk factor for cardiovascular disease which is the leading cause of morbidity and mortality in chronic kidney disease patients [13]. Current literatures have reported that hyperhomocysteinemia is a potential risk factor for venous thrombosis, atherosclerosis and vascular disease [14]. The free radicals produced in homocysteine related pathogenesis causes vascular endothelial cell injury thereby contributing to atherosclerosis, reduction in estimated glomerular filteration rate and further worsening of the disease [23].

D-dimer, a soluble product formed from the degradation of a cross linked fibrin by plasmin during clot breakdown have been documented by various studies as a sensitive marker in coagulation activation and fibrinolysis [24]. Basically, D-dimer indicates the existence of intravascular clot formation and reflects early process of fibrinolytic activities thus playing a crucial role in the diagnosis of thromboembolism. Thromboembolic events are the main factors affecting the prognosis of chronic kidney disease [25]. Reports have shown that a high D-dimer level which is associated with high risk of arterial and venous events in vascular disease are not only specific for thrombus formation but could also be attributed to infection and other miscellaneous inflammatory processes. Therefore, elevated D-dimer level is a prognostic factor for chronic kidney disease and an important marker of fibrin loss in renal insufficiency [26].

1.2 Statement of the problem

Chronic kidney disease is one of the fastest growing public health problem accounting for considerable morbidity and mortality rate with the elderly bearing the greatest burden owing to the underlying risk

factors and co-morbidity. The existing evidence of complications arising from irreversible renal function deterioration in chronic kidney disease patients leading to endothelial dysfunction, hypercoagulability, inflammation, oxidative stress and anaemia has posed a great challenge to both the patients and the health care providers in the management of chronic kidney disease.

1.3 Justification

Chronic kidney disease ranked the 6th fastest growing cause of death worldwide with >10% of the general population affected resulting to at least 2.4 million deaths annually [3]. Though a non-communicable disease, its burden is substantial. This study is aimed at evaluating if the association of homocysteine, interleukins and haematological indices are substantial determinants of renal function deterioration and can independently or collectively predict the risk of chronic kidney disease. The relationship between these parameters are still being explored as there are paucity of data thus, the findings from this study will serve as a reference point for future researches. The understanding of this parameters in chronic kidney disease patients will go a long way to address and prevent the complications as well as assist in the management and treatment of chronic kidney disease patients within Enugu metropolis and the country at large.

1.4 Aim

The aim of the study was to evaluate homocysteine, D-dimer and biochemical parameters in relation to chronic kidney disease stages among patients attending specialist hospitals in Enugu, Enugu State.

1.5 Specific objectives

The specific objectives were:

1. To assess the kidney function among people with chronic kidney disease in Enugu using electrolytes, urea and creatinine test.

2. To determine the homocysteine level among people with chronic kidney disease in Enugu by ELISA technique.

3. To determine the fibrinolytic status among people with chronic kidney disease in Enugu using D-dimer assay by ELISA technique.

4. To determine the relationship between homocysteine, D-dimer and biochemical parameters with stages of chronic kidney disease.

2. Literature Review

2.1 Classification of chronic kidney disease

Assessment of kidney function is achieved using the best indicator, glomerular filtration rate which equates the total amount of fluid filtered through all the functional nephron per unit of time [27]. Glomerular filtration rate decline is dependent on the number of nephrons and the extent of damage on each nephron. End stage renal disease which is the final stage of chronic kidney disease is characterized by glomerular filtration rate less than 15ml/min/1.73m^2 and evidence of less than 10% nephron function left [28]. At this stage, electrolyte and toxin accumulations results to uremic syndrome and could only be managed by kidney replacement, therapy (dialysis or kidney transplant).

National Kidney Foundation and KDIGO 2012 clinical practice guideline developed the classification of chronic kidney disease into five (5) stages for accurate assessment of the disease severity and better provision of management and care for the patients [29]. The stages are determined using estimated glomerular filtration rate and albuminuria (urine albumin creatinine ratio (UACR))

Criteria for chronic kidney disease (either of the following should be present for >3 months)

- Albuminuria (AER ≥30 mg/24 h; ACR ≥30 mg/g [≥3 mg/mmol])
- Urinary sediment abnormality
- Electrolyte and other abnormalities due to tubular disorders
- Abnormalities detected by histology
- Structural abnormalities detected by imaging
- History of kidney transplantation
- GFR <60 mL/min/1.73 m^2

CKD chronic kidney disease, AER albumin excretion rate, ACR albumin-to-creatinine ratio, GFR glomerular filtration rate (Reproduced with permission from Elsevier).

GFR categories in chronic kidney disease

GFR Category	GFR (mL/ min/1.73 m^2)	Terms
G1	≥90	Normal/high
G2	60–89	Mildly decreased (relative to young adult level)
G3a	45–59	Mildly to moderately decreased
G3b	30–44	Moderately to severely decreased
G4	15–29	Severely decreased
G5	<15	Kidney failure

GFR glomerular filtration rate, CKD chronic kidney disease

Albuminuria categories in chronic kidney disease

Category	AER (mg/24 h)	ACR (mg/mmol)	(mg/g)	Terms
A1	<30	<3	<30	Normal to mildly increased
A2	30–300	3–30	30–300	Moderately increased (relative to young adult level)
A3	>300	>30	>300	Severely increased (including nephrotic syndrome)

AER albumin excretion rate, ACR albumin-to-creatinine ratio

The measurement of glomerular filtration rate for stages 1 and 2 chronic kidney disease may not be sufficient for its identification as the patient have normal or borderline normal glomerular filtration rate. Thus, international guideline recommends that the presence of one or more of the following markers of kidney damage can establish the diagnosis [30].

Guideline also recommends the inclusion of estimated glomerular filtration rateand albuminuria levels in evaluating risk for chronic kidney disease, acute kidney injury, end stage renal disease overall mortality and progression of chronic kidney disease

2.2 **Epidemiology**

Chronic kidney disease is one of the most leading causes of morbidity and mortality in this 21st century. Though a non-communicable disease, its burden is substantial. According to 2010 study carried out by the Global Burden of Disease (GBD), chronic kidney disease ranked 27th in the list of causes of total global death in 1990 but rose to 18th in 2010 and 13th by 2013 [4]. This degree of death rate increment is second only to the deaths caused from complications of HIV and AIDS [31]. Currently, chronic kidney disease is the 6th fastest growing cause of worldwide death with >10% of the general population (850 million) affected resulting to at least 2.4 million deaths annually [3]. This substantial rise in the prevalence of chronic kidney disease could be as a result of increased population of aged individuals, Type 2 diabetics mellitus, hypertension, cardiovascular disease and obesity [32].

The incidence, prevalence and progression of chronic kidney disease vary within countries by ethnicity and socioeconomic status [3]. It affects all age groups mostly female in both developed and underdeveloped countries. People in low income countries have 60% greater risk of a progressive chronic kidney disease than the higher socioeconomic quartile due to lack of access to affordable treatment [33]. The prevalence of chronic kidney disease in high income countries like USA and Australia is reported to be 11% [34]. Chronic kidney disease is a growing challenge in low and middle-income countries, particularly in sub-Saharan Africa. A recent systematic review in sub-Saharan Africa reported chronic kidney disease prevalent rate of 13.9% and 10.1% with West Africa having a pooled prevalent rate of 16% which is the highest in the continent [35]. The prevalence is worse in Nigeria and some developing sub-Saharan countries like Chad, Niger and Mali due to demographic and socioeconomic peculiarities thus

exerting a huge economic burden on families and country at large due to increased incidence and heightened morbidity and mortality rate [36].

Despite the huge population of Nigeria with over 180 million people, little is known about the true prevalence of chronic kidney disease in Nigeria [37]. There is insufficient national based data on chronic kidney disease prevalence in Nigeria required to estimate its true burden though most prevalence and incidence statistics are based on community based or institutional studies. This could be due to poor documentation, ignorance, lack of access to health facilities, personal belief or religious inclination [38]. Various hospital based studies have reported chronic kidney disease prevalence in Nigeria of between 1.6% and 12.4% with high prevalence of risk factors among different groups [38]. Oluyombo et al., in a research carried out in a rural community in Southwest Nigeria reported chronic kidney disease prevalence of 18% [39, 40]. Similar research in Southeast Nigeria reported a prevalence of 11.4% in rural and 11.7% in semi urban dwellers [41]. Another study by Ulasi and Ijeoma, using a teaching hospital in Southeast Nigeria as reference highlighted chronic kidney disease enormity, end stage renal disease cases accounted for 8% of the medical admissions and 42% of all renal admissions (42, 43].

The major risk factors of chronic kidney disease in Nigeria include old age, obesity, use of herbal medicine, hypertension, history of DM, alcohol and prolonged use of anti-inflammatory non-steroidal analgesics [38]. Diabetics and hypertension are the two major universal causes of chronic kidney disease though in Sub-Saharan Africa and other developing countries, interstitial nephritis and chronic glomerulonephritis are common causes due to high prevalence of parasitic, bacterial and viral infections present in those countries [33]. Diabetics, which is the chief contributor of chronic kidney disease followed by hypertension accounts for 50-75% of all chronic kidney disease and affects 400 million people worldwide [45]. Environmental pollution of water by heavy metals and soil by organic compound are also implicated in chronic kidney disease. HIV/AIDS infection endemicity in sub-Saharan Africa is one of the co-morbidity of chronic kidney disease as antiretroviral therapies have nephrotoxic effects involving tubular dysfunction and interstitial nephritis [46].

A contributing cause of high morbidity and mortality rate associated with chronic kidney disease is lack of awareness of the disease. Onset of the disease is clinically silent and if not identified and treated, progresses to advanced stages of kidney failure where renal replacement therapy becomes essential for patient survival. More than two million people undergo dialysis or transplantation representing about 10% of patients that requires treatment [33]. About 80% of those receiving kidney failure therapy are from high income countries with the remaining 20% being those in developing countries [34]. The catastrophic cost of renal replacement therapy in Nigeria is alarming leading to over 70% drop out within months of hemodialysis. Chronic kidney disease is indeed a disease condition associated with decreased quality of life, increased healthcare expenditure and premature mortality [47]. Increased awareness of chronic kidney disease is therefore paramount for early intervention and reduced risk of comorbidity and mortality.

2.3 Pathophysiology

The pathological manifestation of chronic kidney disease is the excessive accumulation of extracellular matrix and loss of renal cells. Renal injuries are caused by immunologic reaction (initiated by immune

complexes), endogenous substances like glucose or paraproteins, tissue hypoxia and ischaemia, genetic defect and exogenous substance like drugs. Irrespective of the root cause, renal diseases are characterized by change in morphology comprising renal inflammation, tubulointerstitial fibrosis, glomerulorosis, tubular atrophy and capillary rarefaction [49]. Renal fibrosis which is the final pathologic manifestation of chronic kidney disease represents the unsuccessful wound healing of kidney after chronic sustained injury. Early kidney fibrosis may be reversible as ECM is prone to proteolysis but if fibrosis progresses, it causes matrix stiff which are high resistant to proteolysis leading to organ dysfunction and possibly end stage renal disease [50]. Pathogenesis of renal fibrosis include glomerulosclerosis and interstitial fibrosis.

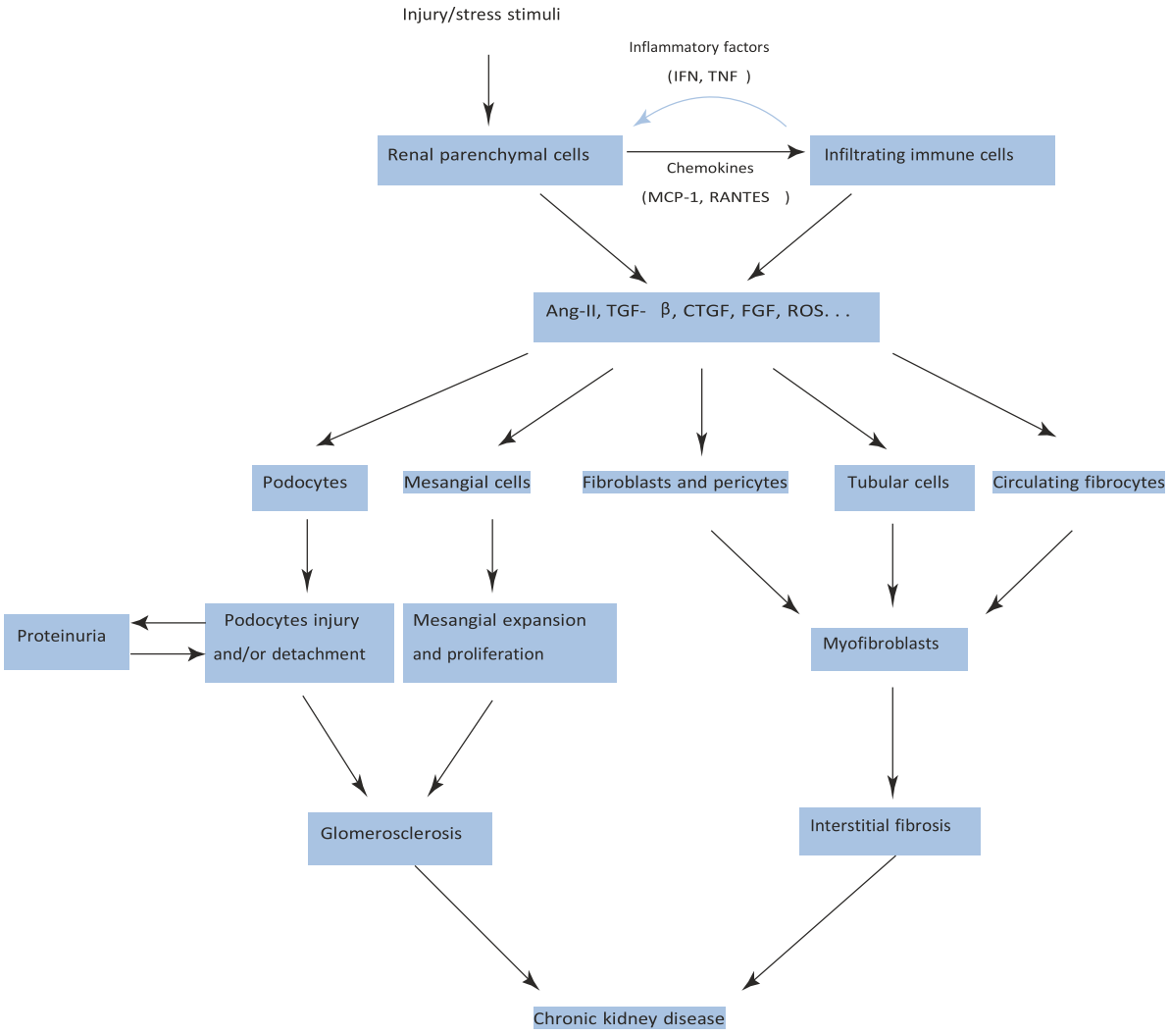


Figure 2.1: Schematic presentation for cellular events involved in the progression of chronic kidney disease

Glomerulosclerosis is prompted by glomerular filtration, proliferation of barrier, proliferation of mesangial cells and destruction of podocytes that line the glomerular basement membrane [51]. In the presence of risk factors like hypertension, dyslipidemia and smoking, glomerulus resident cells are injured and activated leading to the release of cytokines, reactive oxygen species and chemokines which attracts the neutrophils, monocytes, lymphocytes and other inflammatory ells to accumulate at injury site [52]. The infiltrating cells further release cytokines and growth factor like Transforming growth factor β1, Tumor necrotic factor, angiotensin, platelet-derived growth factor, interferon gamma and fibroblast growth factor to induce capillary obliteration, loss of podocytes and parietal epithelium cell activation. Fibrinogenesis (final state) involves production of new extracellular matrix, serving as a baseline for wound closure, remodelling and repair [53]. Podocytes occupy the central role in glomerular disease pathogenesis and its injury may be caused by apoptosis, necrosis and autophagy. Immunoglobin A, Immunoglobin C1 and C3 deposition on the glomerular capillary walls, cytokines or ROS can induce podocyte injury [54]

Renal interstitial fibrosis is characterized by excessive deposition of extracellular matrix in the interstitial compartment prompted by infilterating inflammatory cells, fibroblast activation and myofibroblast development, deposition of extracellular matrix and tubular atrophy with microvascular rarefaction [55]. Interstitial fibrosis, renal atrophy and scaring are closely associated with glomerular filtration rate and proteinuria. At injury like Proteinuria, high glucose conc., resident cells are stimulated to synthesize infiltrating inflammatory compounds including reactive oxygen species, protein factors such as interleukin 1, tumor necrotic factor, angiotension II, necessary for induction of fibroblasts in extracellular matrix production [56]. Activated myefibroblast promotes excessive extracellular matrix deposition in renal interstation. As fibrosis evolves, injured tubular epithelia lose their regenerative capacity and undergo apotosis leading to tubular atrophy and creating non-functional glomeruli.

2.4 Effect of chronic kidney disease on homocysteine

Chronic kidney disease is certainly a growing population globally with the people affected being predisposed to serious cardiovascular risk. Though caused by a heterogeneous range of diseases, one of the possible culprit could be in the marked derangement of a complex sulfur metabolism peculiar to these patients resulting to renal function deterioration. Homocysteine of all the amino acid abnormalities present in chronic kidney disease has attracted much attention due to its proposed role in the development and/or progression of atherothrombotic diseases.

Homocysteine, a sulfhydryl containing amino acid generated by the demethylation of methionine in methionine metabolism and physiological essential for processes like cell cycle progression and maintenance of cellular homeostasis have been implicated in the development and progression of renal diseases [18]. The kidney plays crucial role in the amino acid metabolism and clearance though in renal function impairment, it may compromise in plasma homocysteine clearance and metabolism leading to its accumulation. Renal function is therefore an important determinant of homocysteine level. According to previous research, excessive accumulation of homocysteine causes glomerular cell damage and impairment of renal microvasculature which in turn, further increases homocysteine level leading to chronic renal failure [19].

The plasma level of homocysteine is controlled through two metabolic pathways; remethylation of homocysteine to methionine or transulfuration of homocysteine to cysteine with both pathways requiring the presence of vitamin derived cofactors including pyridoxine (vitamin B6), folate (vitamin B9) and cobalamin (vitamin B12) for the processes [57]. Growing evidence has reported hyperhomocysteinemia as a common feature in chronic kidney disease patients. The underlying pathological mechanism for this phenomenon is not well elucidated but could be due to defective renal clearance of homocysteine and impaired non-renal disposal. Also, total or relative deficiencies of folate, vitamin B12 and vitamin B6 may play a role.

Rapidly accumulating evidence have documented that elevated homocysteine level emanating from nutritional, metabolic or genetic origin is an independent risk factor of thromboembolism [58]. Hyperhomocysteinemia has been shown by ample research to cause endothelial cell damage and increased production and activities of coagulation factors thereby enabling interaction with platelet, clotting factors and lipid hence its important role in thrombosis especially in end stage renal disease [21]. Epidemiological studies have attributed elevated homocysteine level to promote thrombosis through several mechanisms such as increased platelet reactivity, enhanced expression of tissue factor, increased generation of thrombin, reduced anticoagulant (protein C and protein S) processes, impaired fibrinolytic activities, enhanced factor V activities and vascular injury encompassing endothelial dysfunction [59].

Growing evidence over two decades have reported that hyperhomocysteinemia may be an independent risk factor of venous thrombosis, atherosclerosis and vascular disease. However, the molecular mechanism involving hyperhomocysteinemia effect in vascular injury and thrombi formation are not well understood though may be related to hypomethylation of DNA, oxidative stress and proinflammatory effect [60]. Homocysteine plasma concentration of 5-15umol/l in healthy individuals reaches 100umol/l in severe hyperhomocysteinemia. In renal patients, administration of high dose of folic acid reduces plasma homocysteine concentration however, in 50% of cases homocysteine remains above 15umol/l thus the vitamin effect is unclear in homocysteine reduction [61].

Elevated plasma level of homocysteine is also noted as an independent risk factor for cardiovascular disease in chronic kidney disease patients especially in end stage renal disease with the cardiovascular events incidence more heightened in chronic kidney disease patients as compared to the general circulation [13]. Studies have shown that cardiovascular disease is the leading cause of hospitality and a predictor of high mortality rate in chronic kidney disease with stage 3 chronic kidney disease patients more likely to die of cardiovascular disease before progressing to end stage renal disease. Also, cardiovascular disease mortality rate is about 10-20 times higher in end stage renal disease patients than the general population [62]. In chronic kidney disease, the given therapies targeted to slow its progression to end stage renal disease are the ACE inhibitors and angiotensin II receptor block for their action as an antihypertensive, anti-inflammatory and anti-fibrosis. Hyperhomocysteinemia are therefore independently associated with cardiovascular events comprising thromboembolism and atherosclerotic arterial disease like myocardial infarction and stroke [13]. Giving the relationship between homocysteine and cardiovascular disease, it remains unclear whether treatments with vitamins directed to reduce homocysteine level could decrease the risk of arterial and venous thromboembolic events.

Chronic kidney disease defined as the structural defect or decreased function of the kidney is diagnosed by having at least one of the following criteria; glomerular filtration rate of <60ml/min/1.73m^2, albuminuria, renal tubular disorder or imaging evidence of renal injury. Plasma homocysteine have been stated by literatures to have a strong correlation with glomerular filtration rate as reduced glomerular filtration rate increases circulating levels of homocysteine in chronic kidney disease patients though dependent on the severity of renal impairment. An experimental study demonstrated that hyperhomocysteinemia was decreased after kidney transplantation consequently, it is documented that renal mechanisms inevitably are responsible for elevated homocysteine levels in chronic kidney disease patients [63].

2.5 **Effect of chronic kidney disease on D-dimer**

D-dimer, a soluble product formed from the degradation of cross-linked fibrin by plasmin during clot breakdown have been documented by various studies as a sensitive marker in coagulation activation and fibrinolysis [24]. Basically, chronic kidney disease patients are known to have coagulation disorders with resulting thrombotic complication being the most common cause of mortality and posing a great difficulty in renal replacement therapy among chronic kidney disease patients [21]. The coagulation process literally involves the interaction between the platelets, coagulation system, vascular endothelium, anticoagulant system and fibrinolytic system.

D-dimer are proteins that indicates the existence of intravascular clot formation and reflects the early process of fibrinolytic activities thus playing a crucial role in the diagnosis of thromboembolism [25]. Thromboembolic events have been stated by various literatures as a crucial factor affecting the prognosis of chronic kidney disease patients. Haemostatic alterations could possibly contribute to this heightened risk of thrombotic events though their roles remain elusive. Studies have shown that patients with chronic kidney disease exhibits endothelial cell dysfunction and increased coagulation activities mostly factor 8 [64].

According to growing evidence, renal failure has been associated with elevated levels of D-dimer not only due to decreased D-dimer elimination by the kidneys but also due to coagulation activation in patients with renal disease [26]. Literatures have also documented that decreased renal function seen in chronic kidney disease patients is associated with increasing levels of other haemostatic markers such as fibrinogen, soluble thrombomodulin, factor viii levels and thrombin-antithrombin complex. Increased plasma level of D-dimer is recorded in the presence of a clot but also in other medical situation requiring coagulation system activation. Therefore, D-dimer is not a specific marker of thrombosis.

D-dimer plasma level in a healthy individual is low with its elevation as an indicator of increased fibrinolysis termed by previous researches as a classic hypercoagulability biomarker useful in the diagnosis of thromboembolic events [65]. D-dimer levels tend to increase as the renal disease progresses in chronic kidney disease patients pointing that the hypercoagulability state could be an existing link between chronic kidney disease and elevated risk of cardiovascular outcomes. Current studies have linked D-dimer levels with the development of atherothrombosis and cardiovascular events in chronic kidney disease patients thus insinuating that D-dimer could be important in evaluating cardiovascular disease risk

in these patients [66]. In end stage renal disease patients under hemodialysis, D-dimer assessment tends to have no definite diagnostic value accruing to the existence of several other comorbidity like atherosclerosis or malignancy in these patients which also are associated with elevated plasma D-dimer levels.

Owing to the high sensitivity and negative predictive value, assessment of D-dimer is routinely used in medical practice as the primary baseline test for patients being suspected with venous thromboembolism. Current research has shown that the risk of thromboembolism is heightened across the spectrum of chronic kidney disease including mild and more advanced chronic kidney disease, nephrotic syndrome and after kidney transplantation [65]. The underlying mechanism resulting to this risk increment have not been well understood but could be due to activation of procoagulants, enhanced platelet activation and aggregation, decreased endogenous anticoagulants and decreased fibrinolytic activities. D-dimer which is associated with high risk of arterial and venous events are not only specific for thrombi formation but could also be attributed to infection and other miscellaneous inflammatory processes [67].

3. Materials and Methods

3.1 Study design

This research adopted a cross-sectional study design. A hospital based study focused on subjects between 18-65 years of age. Participants were recruited from already diagnosed chronic kidney disease patients attending nephrology outpatient clinic and on admission at Enugu State University Teaching Hospital, Parklane and University of Nigeria Teaching Hospital, Ituku-Ozalla. The control group were apparently healthy individuals, age and sex matched using same selection and evaluation process. This study ran from May, 2025 to September, 2025 and the subjects' demographic and clinical details were obtained using a well-structured questionnaires.

3.2 Study Area

Enugu State was created on 27th August, 1991 and it is situated in the South-East geographical zone of Nigeria. It covers an area of 7,161km^2, located at 63^{o}N of the equator and 73" of latitude. It has 17 local government areas with an estimated population of 3,269837 in which 1,596,042 are males and 1,671795 are females (68). Geographically, it is located at the foot of Udi Plateau in the basin of Cross River and the Benue trough. The state harbours different ethnic groups though predominantly occupied by Igbos mainly civil servants, students, business enterprise and farmers. The climatic condition of the state is divided into wet season; which runs between the period of April to September and dry season; which is between October and March. It shares borders with the following States; Abia and Imo to the South, Ebonyi to the East, Kogi to the North-West and Anambra to the West. Enugu state is the home of many polytechnics, colleges of education, universities including University of Nigeria, Nsukka. It has health facilities like University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu State University Teaching Hospital, Parklane and other government owned/private establishments around the State.

3.3 Sample Size

The sample size, n for the study was calculated using the fomular below

$$n = \frac{Z^2(P)(1-P)}{d^2}$$

where

n = the desired sample size when the population is more than 10,000

Z = standard deviation usually set at 1.96, which corresponds to 95% confidence interval.

P = proportion in the target population estimated to have a particular characteristic. (Prevalence of CKD in a Semi-Urban community of South-East, Nigeria by (Okwuonu et al, 2017) was 0.078

d = degree of accuracy desired set at 5% (that is tolerated error of 5%) which is equal to 0.05.

Therefore, the minimum sample size,

$$n = \frac{1.96^2(0.078)(1-0.078)}{0.05}$$

$$n = \frac{3.8416 \times 0.078 \times 0.922}{0.0025}$$

$$= 110$$

Plus 10% attrition rate = 121

3.4 Ethical Consideration

Ethical clearance was obtained from the health research and ethical committee of the University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu State. Detailed explanation of the aims and benefits of this research was given to each participant. Informed consent was obtained from the subjects and caregivers before administration of the questionnaire and collection of blood sample.

3.5 Study Population

A total of one hundred and eighty subjects both males and females between 18 - 65 years of age were used for this study. One hundred subjects were already diagnosed and classified adults infected with chronic kidney disease attending Enugu State University Teaching Hospital, Parklane and University of Nigeria

Teaching Hospital, Ituku-Ozalla, Enugu. Eighty were apparently healthy subjects not diagnosed with chronic kidney disease within same facilities, sex and age matched.

3.6 Questionnaire

A well-structured close ended questionnaire was issued to each participant of the target population to obtain their demographic data and clinical history.

3.6.1 Inclusion criteria

Inclusive subjects were male and female between 18-65 years of age who had given their informed consent and those who were diagnosed and classified chronic kidney disease patients.

3.6.2 Exclusion criteria

Subjects who refused to give their informed consent were excluded from this study. Subjects with cancer or sickle cell were excluded. Pregnant women and lactating mothers were also excluded from this research work.

3.7 Sample Collection

A total of 5mls of whole blood sample was collected by venepuncture under asceptic conditions from the consented participants, 2ml was transferred into sodium citrate container for D-dimer assay and the remaining 3ml into plain tube for biochemical tests. Both platelet poor plasma and serum were obtained by centrifugation at 3000rpm for 15mins for quantitative D-dimer, homocysteine, electrolytes, urea and creatinine assay.

3.8 Laboratory Procedure

Quantitative evaluation of urea and creatinine was done using biochemical auto analyser, Cobas 111, electrolytes was done using EXIAS auto analyser while homocysteine and D-dimer assay was done using ELISA technique The procedure for the assay was followed strictly according to the manufacturer's manual.

Principle of urea: Urea is measured by Berthelot reaction. Serum urea is hydrolysed to ammonia in the presence of urease. The ammonia reacts with hypochlorite and phenol to give indophenols (blue compound), which is then measured photometrically by Bertholet reaction.

$$\text{Urea} + H_20 \leftrightarrow 2NH_3 + CO_2$$

$$NH_3 + \text{hypochlorite} + \text{phenol} \rightarrow \text{phenol indole (blue compound)}$$

Principle of creatinine: Creatinine was estimated using Jaffe reaction. Creatinine in alkaline solution reacts with picric acid to form a coloured complex (yellow orange complex). The intensity of the colour

produced is directly proportional to the amount of creatinine in the samples which is determined photometrically.

Principle of Homocysteine Assay: The principle is based on competitive enzyme immunoassay technique utilizing a polyclonal anti - HCY antibody and an HCY-HRP conjugate. The assay sample and buffer is incubated together with HCY-HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of enzyme - substrate reaction forms a blue coloured complex. Finally, a stop solution is added to stop the reaction which will then turn the solution yellow. The intensity of the colour measured spectrophotometrically at 450nm in a microplate reader. The intensity of the colour is inversely proportional to the HCY concentration since HCY from the samples and HCY-HRP conjugate compete for the anti-HCY antibody binding sites. Since the number of sites are limited, as more sites are occupied by the HCY from the sample, fewer sites are left to bind HCY-HRP conjugate. A standard curve is plotted relating the intensity of the colour to the concentration of standards. The HCY concentration in each sample is interpolated from the standard curve.

Principle for the D-dimer assay: The human D-Dimer solid phase sandwich ELISA is designed to quantitatively measure the amount of the target bound between antibody pairs. Target-specific antibody has been pre-coated in the wells of the provided micro-plates. Samples, standards or controls are added into these wells and binds to the immobilized (captured) antibody. The sandwich is formed by addition of second (detector) antibody, a substrate solution is further added which reacts with the enzymes-antibody target complex to produce measurable signal. The intensity of this signal is directly proportional to the concentration of the D-Dimer present in the human plasma or serum.

3.9 Statistical analysis

Data were analyzed using Graph pad prism software version 9.3.1 and SPSS software version 25. Quantitative independence variables data were expressed as standard error of mean ± (SEM) and categorical variables such as age, sex, clinical history, stages of disease were presented as proportion using tables and unpaired t-test. Comparism of categorical variables between the two groups (study group and control group) was done using chi-square and continues variables, mean difference between two groups were analyzed using student t square test. Comparism between more than two groups was done using ANOVA. Data were subjected to descriptive statistics and analyzed using analysis of variance and student's T-test. The probability value less than 0.05 were considered statistically significant.

4. Results

4.1 General characteristics of the study subjects

A total of one hundred and eighty subjects were enrolled in this study. One hundred were chronic kidney disease (CKD) patients comprising of 57% (57/100) and 43% (43/100) males and females respectively, with mean age of 43.08 ± 13.32 years old. Apparently healthy individuals without kidney problem were enrolled and they comprised of eighty subjects, consisting of 57.5% (46/80) and 42.5% (34/80) males and females respectively. The mean age of the control subjects was 39.08 ± 13.11 years old.

The age group of 31-40 years had the highest frequency with 23% amongst the test group while the age of 21-30 years with 25% were the majority in the control group. The age group of 18-20 years and 61-65 years amongst the study group were 3% and 13% respectively as against the control group with 6.3% each in the same group. Majority of both the test group and controls were married, accounting for 75% (75/100) and 63.8% (51/80) respectively.

The assessment of comorbidities indicated that some of the subjects 54%, 24% and 3% were suffering from hypertension, diabetes and cardiovascular disease respectively. Some of the patients had both hypertention and diabetes and accounting for 9% while 10% of the study subjects had nephritis

Table 4.1 General characteristics of the study subjects

Variable	**Test group (n=100)** **f (%)**	**Controls (n=80)** **f (%)**
Gender:		
- Male	57 (57)	46 (57.5)
- Female	43 (43)	34 (42.5)
Age (years):		
18- 20	3 (3)	5 (6.3)
- 21- 30	21 (21)	22 (27.5)
- 31 – 40	23 (23)	18 (22.5)
- 41 - 50	21 (21)	16 (20.0)
- 51 - 60		
- 61 - 65	19 (19)	14 (17.5)
	13 (13)	5 (6.3)
Marital status:		
- Single	25 (25)	29 (36.3)
- Married	75 (75)	51 (63.8)
Co-morbidity		
- Hypertension	54 (54)	-
- Diabetics	24 (24)	-
- Hypertension/Diabetes	9 (9)	-
- Nephritis	10 (10)	-
- Cardiovascular disease	3 (3)	-

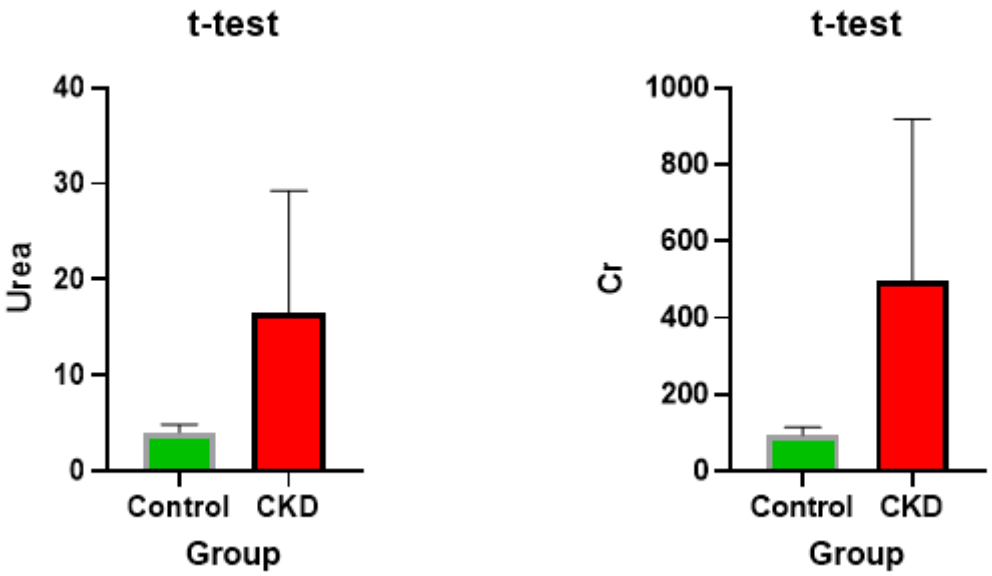
4.2 Estimation of Kidney function markers and electrolyte values

The electrolytes result showed that sodium, chloride and bicarbonate had a significantly lower ($p = 0.001$, $p = <.001$ and $p = 0.005$ respectively) values in CKD patients with mean 133.44 ± 15.652 mmol/l, 100.44 ± 6.321 mmol/l and 22.16 ± 2.638 mmol/l respectively as compared to 139.41 ± 4.371mmol/l, 104.92 ± 3.785 mmol/l and 23.38 ± 3.18 mmol/l respectively seen in the controls while potassium levels were higher in the CKD group but not statistically significant ($p = 0.08$).

The kidney function markers, Urea and creatinine were significantly increased (p = <.001) in CKD patients with mean values of 16.47 ± 1.79 mmol/l and 497.19 ± 42.08 µmol/l respectively compared to 4.04 ± 0.86 mmol/l and 94.24 ± 20.24 µmol/l in controls respectively.

Table 4.2 Estimation of Kidney function markers and electrolyte values among the CKD patients and controls.

Parameter	Test group (n =100)	Control (n=80)	P-value
	Mean ± SD	Mean ± SD	
Sodium (mmol/l)	133.44 ± 15.652	139.41 ± 4.371	0.001
Potassium (mmol/l)	5.04 ± 6.375	3.78 ± 0.538	0.08
Chloride (mmol/l)	100.44 ± 6.321	104.92 ± 3.785	<.001
Biocarbonate (mmol/l)	22.16 ± 2.638	23.38 ± 3.18	0.005
Urea (mmol/l)	16.47 ± 1.794	4.04 ± 0.855	<.001
Creatinine (µmol/l)	497.19 ± 42.081	94.24 ± 20.236	<.001



Graph showing comparison of creatinine level of people with CKD and controls

4.2.1 Classification of kidney function markers and electrolyte values

The frequency of the kidney function markers and electrolytes values in CKD patients revealed that those with normal sodium values were 51%, 41% had low while 8% had high values. Most of the patients had normal result for potassium, chloride and bicarbonate, 75%, 77% and 73% respectively, few had low 11%, 17% and 27% respectively while 14% and 6% had high value result in potassium and chloride respectively.

Majority of the chronic kidney disease patients had high values of urea and creatinine, 83% and 89% respectively while those who had normal values were 17% and 11% respectively.

Table 4.2.1 Classification of kidney function markers and electrolyte values among CKD patients

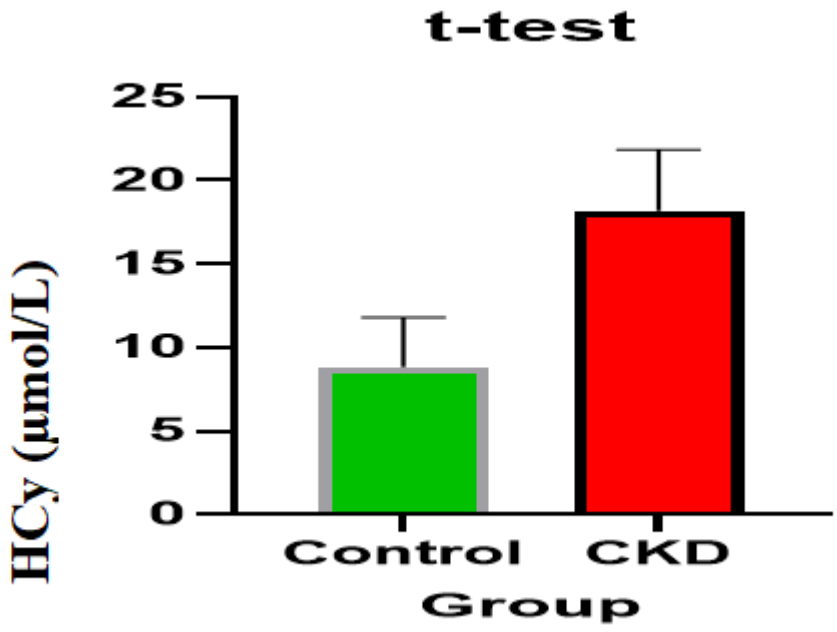
Value of parameters	f (%)	Range	M±SD
Sodium		101.0-150.0	134.81±8.19
- Low	41 (41)		128.06±8.02
- Normal	51 (51)		138.23±2.40
- High	8 (8)		147.60±1.44
Potassium		2.10-7.60	4.19±0.81
- Low	11 (11)		2.90±0.41
- Normal	75 (75)		4.12±0.40
- High	14 (14)		5.56±0.69
Chloride		75.0-113.0	100.44±6.32
- Low	17 (17)		89.65±6.16
- Normal	77 (77)		102.00±2.57
- High	6 (6)		111.00±1.26
Bicarbonate		12.50-30.00	22.16±2.64
- Low	27 (27)		19.13±2.23
- Normal	73 (73)		23.28±1.75
Urea		3.60-69.40	16.47±12.79
- Normal	17 (17)		6.44±1.47
- High	83 (83)		18.53±13.12
Creatinine		124.70-2001.00	497.19±420.81
- Normal	11 (11)		167.44±18.40
- High	89 (89)		537.95±428.84

4.3 **Estimation of Homocysteine and D-dimer levels among chronic kidney disease patients and controls**

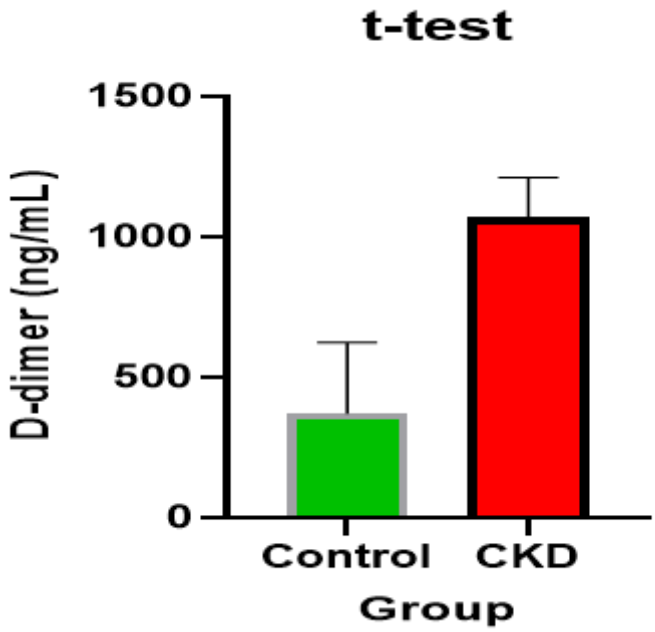
Homocysteine and D-dimer levels were significantly increased (p = <.001) among the CKD patients presenting with mean of 18.20 ± 3.63 µmol/l and 1069.02±143.26 pg/ml respectively as compared to 8.85 ± 2.96 µmol/l and 370.78±253.34 pg/ml in the control group.

Table 4.3 Estimation of Homocysteine and D-dimer among the chronic kidney disease patients and controls.

Parameter	**Test group (n =100)**	**Control (n=80)**	**P-value**
	Mean ± SD	**Mean ± SD**	
Homocysteine (µmol/l)	18.20±3.64	8.85±2.96	<.001
Dimer (pg/ml)	1069.02±143.26	370.78±253.34	<.001



Graph showing Comparison of homocysteine levels of chronic kidney disease patients and controls



Graph showing comparison of D-dimer levels of chronic kidney disease patients and control

4.3.1 **Classification of the biochemical markers among the CKD patients**

The values of the parameters assessed in patients with CKD is grouped in relation to their normal ranges to ascertain the frequency of the results gotten. Most of the CKD patients examined had high homocyteine and D-dimer levels accounting for 85% and 82% respectively with 15%, 18% being those with normal.

Table 4.3.1 Classification of the biochemical markers among the CKD patients

Parameters	f (%)	Range	M±SD
Homocysteine		8.92-25.71	18.20±3.64
- Normal	15 (15)		12.36±2.02
- High	85 (85)		19.23±2.78
D-dimer		237.0-1301.0	1069.02±143.26
- Normal	18 (18)		868.27±171.84
- High	82 (82)		1113.09±89.25

4.4 Classification of CKD patients in relation to estimated glomerular filteration rate

The table below shows the distribution of respondents by stages of CKD among the test group. The estimated glomerular filtration rate of CKD patients enrolled in this study was within the range of 2-47 with mean of 19.40±12.78. 29% of patients were in stage 3 (mild to moderate stage) with mean 36.39±4.84, comprising of 3% in stage 3a with mean 45.67±1.15 and 26% in stage 3b with mean of 35.28±3.77. Stage 4 (moderate to severe stage) comprised of 25% of the patients with mean 21.62±4.51 while stage 5 (end stage renal disease) was 46% with mean of 7.80±3.77.

Table 4.4 Classification of chronic kidney disease patients in relation to estimated glomerular filtration rate (eGFR)

Parameters	f (%)	Range	M±SD
eGFR		2-47	19.40±12.78
- Stage 3	29 (29)		36.39±4.84
Stage 3a	3		45.67±1.15
Stage 3b	26		35.28±3.77
- Stage 4	25 (25)		21.62±4.51
- Stage 5	46 (46)		7.80±3.77

4.5 Correlation of estimated glomerular filtration rate stages with homocysteine and D-dimer

There was a statistically significant variation in plasma homocysteine levels across the stages of chronic kidney stages (p = 0.009). A systematic increase was recorded, with the patients in stage 5 (end-stage) having the highest mean homocysteine concentration. In this study, Stage 3, stage 4 and stage 5 of chronic kidney disease patients recorded homocysteine mean values of 17.14±3.14 µmol/l, 17.24±3.18 µmol/l and 19.40±3.86 µmol/l respectively.

D-dimer levels across different stages of chronic kidney disease among the study participants also recorded statistically significant progressive difference (p = 0.024). Patients in stage 5 recorded the highest mean D-dimer level of 1103.18 ± 121.22 ng/ml, followed by stage 4 with mean of 1071.10 ± 198.12 ng/ml and stage 3 having the lowest mean D-dimer level of 1013.05 ± 100.70 ng/ml

Table 4.5 Assessment of estimated glomerular filtration rate stages with homocysteine and D-dimer

	Stage 3 (n = 29)	Stage 4 (n = 25)	Stage 5 (n = 46)	p-value
Homocysteine (µmol/l)	17.14±3.14	17.24±3.18	19.40±3.86	.009

D-Dimer (ng/ml)	1017.95±98.97	1063.59±197.85	1103.18±121.22	.024

Correlations analysis between homocysteine, D-dimer, estimated glomerular filtration , electrolytes and kidney function markers

The correlation analysis presented in the table below examined the relationships between homocyteine, D-dimer, estimated glomerular filteration rate, electrolytes, urea and creatinine among people with chronic kidney disease (CKD).

The results revealed that homocysteine showed a significant positive correlation with creatinine (r = 0.259, p = 0.009) and D-dimer(r = .222, p = .027) and a significant negative correlation with estimated glomerular filtration rate (r = -.250, p = .012). Although the correlation between homocysteine and urea was positive (r =.174), it did not reach statistical significance (p = 0.083).

The correlation of D-dimer with other variables showed that it had a significant positive correlation with homocysteine (r = .222, p = .027) and a significant negative correlation with estimated glomerular filtration rate (r = -.258, p = .009). Though it had a positive correlation with creatinine and urea, the associations were not statistically significant.

Tale 4.6 Correlations analysis between homocysteine, D-dimer, electrolytes and kidney function markers.

Correlation		eGFR	HCy	D-dimer	Na	K	Cl	HCO_3	Ur	Cr
HCy	Pearson Correlation	-.250*		.222*	-.030	-.154	-.098	-.031	.174	.259**
	Sig. (2-tailed)	.012		.027	.765	.126	.331	.762	.083	.009
	N	100		100	100	100	100	100	100	100
D-dimer	Pearson Correlation	-.258*	.222*		-.166	.023	-.128	-.077	.100	.174
	Sig. (2-tailed)	.009	.027		.099	.818	.206	.444	.323	.083
	N	100	100		100	100	100	100	100	100
eGFR	Pearson Correlation		-.250*	-.258*	.182	-.322*	.162	.159	-.588**	-.741**
	Sig. (2-tailed)		.012	.009	.069	.001	.107	.114	<.001	<.001
	N		100	100	100	100	100	100	100	100

4.7 Logistic regression analysis of predictors of Chronic Kidney Disease Stages

The multinomial logistic regression analysis demonstrated a significant relationship between several biochemical and hematological parameters with the progression of chronic kidney disease stages. Renal function markers such as urea, creatinine, and estimated glomerular filtration rate (eGFR) showed strong associations with CKD stages.

Electrolyte particularly elevated potassium and reduced bicarbonate levels, were also significant. Increased levels of D-dimer and homocysteine were significantly associated with higher CKD stages.

Table 4.7 Multinomial Logistic Regression Analysis of homocysteine, D-dimer, electrolytes, urea and creatinine predictors of Chronic Kidney Disease Stages

Variables	B (Stage vs 3)	4p-value	Exp(B)	B (Stage 5 vs 3)	p-value	Exp(B)
Constant	-8.12	0.021	—	-10.34	0.009	—
Urea (mmol/L)	0.087	0.002	1.091	0.143	0.001	1.154
Creatinine (µmol/L)	0.019	0.01	1.019	0.034	0.000	1.035
Sodium (mmol/L)	-0.042	0.082	0.959	-0.05	0.064	0.951
Potassium (mmol/L)	0.214	0.11	1.239	0.357	0.044	1.429
Chloride (mmol/L)	0.018	0.295	1.018	0.022	0.221	1.022
HCO_3 (mmol/L)	-0.073	0.038	0.93	-0.109	0.021	0.896
D-dimer (ng/mL)	0.004	0.049	1.004	0.007	0.015	1.007
Homocysteine (µmol/L)	0.059	0.051	1.061	0.086	0.029	1.09

Chapter five

Discussion and Conclusion

The kidney as an important organ of man and animals is very vital for human health and existence. Kidney dysfunction caused either due to life style activities such as indulging in recreational drugs or microbial

infections such as viral hepatitis can lead to renal impairment and this will result to non-excretional or filteration activities of the kidney thereby leading to the accumulation of waste [70].The kidney disease is classified into the acute phase and chronic phase but none of them is palatable for human health. In chronic kidney disease, patients are always administered with dialysis to remove accumulated waste. This therapy and medication is very expensive and involves a breakdown of body molecules and ions. The outcome of non-functional biomolecules and ions is wasting of such individual and eventually death [71].

In typical traditional system, the onset of kidney problems are not diagnosed as the individuals resort to suspicions of witch hunting and disease transfer from either family members or neighbours thereby leading to consumption of herbal concoctions. This will result to late reporting to the hospital which eventually further affect the kidney and increase the disease burden. Therefore, this study was aimed to determine the changes in selected biomolecules such as homocysteine and D-dimer among the patients with chronic kidney disease. The study also assessed the kidney function markers among these patients in relation to the impact of chronic kidney disease.

The demographic profile of the study participants reveals a critical aspect of CKD epidemiology. A slight male predominance was observed in both test (57%) and control (57.5%) groups indicating that the males were more affected than the females in this study. This findings is consistent with existing literature that suggests a higher susceptibility or progression rate of chronic kidney disease in males, potentially influenced by hormonal factors, lifestyle or differences in the prevalence of underlying conditions like hypertension [72]. The near-identical gender ratio between groups strengthens the comparability of the control and test cohorts for the study's objectives.

The age distribution shows that 25% of the test group between 31-40 years and 27.5% of the control between 21-30years age bracket were the predominant group though there was a notable presence of chronic kidney disease patients within 41-65 years range. This aligns with the global burden of chronic kidney disease, which affects a significant proportion of the working-age adult population, contributing substantially to morbidity and mortality [73]. The higher percentage of end-stage cases in the older age groups (61-65 years) underscores the progressive nature of the disease and its increased prevalence with advancing age. This distribution indicates that middle-aged adults constituted the majority of the study participants.

Married individuals formed a larger proportion of the respondents in this study. While not a direct risk factor, this demographic characteristic may have implications for social support systems, healthcare-seeking behavior, and the psychosocial burden of managing a chronic illness like chronic kidney disease. The reasons behind this distribution warrant further socio-epidemiological investigation.

Analysis of the co-morbidity/risk factors among the test group unequivocally identifies hypertension (54%) and diabetes (24%) as the primary etiologies for chronic kidney disease in this population thus highlighting their major role in kidney damage and impaired renal function. This finding is consistent with global patterns where these two conditions are the leading causes of end-stage renal disease [74, 75]. The significant overlap, with 9% of patients having both conditions, highlights a synergistic detrimental effect on renal function, a phenomenon noted in other studies [76]. The presence of nephritis and cardiovascular

disease as additional risk factors further underscores the multifactorial nature of chronic kidney disease, where both metabolic and inflammatory processes contribute to disease.

The distribution of disease stages presents a concerning clinical picture, with nearly half (46%) of the CKD patients already at the end-stage. This high percentage suggests issues related to late presentation, delayed diagnosis, or inadequate control of underlying risk factors in the early stages of the disease. This aligns with concerns raised in other studies, particularly in regions where access to early screening and nephrology care may be limited [77].

The analysis of electrolytes and renal function tests provides direct evidence of the kidneys' excretory failure and inadequacies in the regulation of extracellular fluid volume. The dramatic elevations in urea and creatinine recorded in this study are direct consequences of a reduced glomerular filtration rate (GFR), confirming the severity of renal impairment in the treatment group [78]. These markers are the cornerstone of chronic kidney disease diagnosis and staging. The logistics regression analysis of biochemical parameters with chronic kidney disease stages in this study showed that urea and creatinine are predictors of chronic kidney disease. Current studies have shown that the rapid elevation of waste products; urea and creatinine occurs when the kidney function might have dropped to 50% [79, 80]. El-ishag et al, (2021) in an observational study conducted in a specialist hospital in Yobe state also reported an increased urea and creatinine in chronic kidney disease patients.

Electrolyte imbalance is one of the leading complications in chronic kidney disease as one of the primary functions of the kidney is to maintain different electrolytes in an acid-base balance. Decreasing renal function certainly causes a progressive increase in acid retention thereby leading to serious consequences like protein energy wasting [7]. The electrolyte imbalances, including hyponatremia, hypochloremia, and a trend towards metabolic acidosis (reduced bicarbonate) recorded in this study are common complications in advanced chronic kidney disease. Hyponatremia occurs due to the kidneys' lost ability to maintain fluids through excretion of sodium leading to fluid retention and subsequently hypertension, electrolyte imbalance and edema [8]. Hypochloremia is not necessarily a direct consequence of renal disease but could be caused as a result of thiazide diuretics prescribed to chronic kidney disease patients for the management of fluid overload and also gastrointestinal loss mostly due to prolonged vomiting and diarrhea [81].

Metabolic acidosis is the acid build up that occurs due to impaired kidneys' ability to excrete acid and regenerate bicarbonate. A functional kidney converts glutamine to bicarbonate and ammonium to help maintain the acid-base balance [82]. The reduced bicarbonate recorded in this study is a clear indication of the presence of metabolic acidosis in chronic kidney disease patients. Thus, there is a direct correlation between declined glomerular filtration rate and reduced serum bicarbonate in chronic kidney disease patients overly caused by decreased ammonia production, impaired regeneration of bicarbonate and reduced hydrogen ions excretion [11]. The trend towards hyperkalemia, though not statistically significant ($p = 0.08$) in this study, remains a critical, life-threatening concern in chronic kidney disease management. Hyperkalemia, a frequently recorded complication in chronic kidney disease is caused by reduced excretion of renal ions as well as medications (renin angiotensin aldosterone system inhibitor drugs) prescribed to them for its beneficial cardio-renal properties [83]. Reports has shown that elevated serum

potassium predisposes to greater risk of end stage renal disease, arrhythmias and mortality. Fukasawa et al, (2022) in a cross sectional study also recorded increased urea, creatinine and hyperkalemia.

The biochemical analysis provides robust evidence of homocysteine kidney marker dysregulation in chronic kidney disease. The observed significantly elevated homocysteine levels in this study (18.20 µmol/L vs. 8.85 µmol/L) is a classic finding suggesting renal impairment and may serve as a potential biochemical marker of chronic kidney disease progression. As demonstrated by Yassin et al, homocysteine accumulates due to impaired renal clearance and increased metabolic disturbances characteristics of the disease [84]. This finding conforms to other research works [75, 76]. Xiao et al, (2021) in a community based cohort study in Beijing, China using one thousand four hundred and twenty six participant also reported an elevated homocysteine levels and further observed the relationship between increased homocysteine level and accelerated decline of estimated glomerular filtration rate. Hyperhomocysteinemia is not merely a marker but also implicated as an independent risk factor of cardiovascular complications, a leading cause of death in this chronic kidney disease patient [13].

The major site of homocyteine metabolism and excretion is through the kidneys. In renal clearance impairment, serum level of homocysteine increases leading to hyperhomocysteinaemia which is toxic to the body. According to previous research, excessive accumulation of homocysteine causes glomerular cell damage and impairment of renal vasculature which in turn further increases homocysteine levels leading to chronic renal failure; such a vicious cycle [19]. Pearson's correlation showed that homocysteine had a significant positive correlation with creatinine in this study suggesting that higher homocysteine levels are associated with increased serum creatinine concentration. In renal function impairment (indicated by increased creatinine level), the kidneys ability to clear homocysteine is compromised leading to the accumulation of both substances in the blood.

The significantly progressive increase in homocysteine levels with disease severity recorded in this study suggests that worsening renal function is accompanied by impaired clearance of homocysteine resulting to its accumulation in the circulation. In multinomial logistic regression analysis done, homocysteine was significantly associated with higher chronic kidney disease stages, indicating that systemic inflammation become more pronounced as renal impairment worsens. Existing literature has documented homocysteine levels correlation to the severity of kidney damage with those in end stage renal disease having significantly higher homocysteine than the initial stage [63]. This significant association between elevated HCy and advanced CKD stages underscores its potential utility as a biochemical marker of disease progression and metabolic dysfunction.

Pearson's correlation also showed significant negative correlation between homocysteine and estimated glomerular filtration rate ($r = -.250$, $p = .012$) suggesting that declining glomerular filtration rate is accompanied by increasing homocysteine levels. Homocysteinemia which is defined as total plasma homocysteine level of above 15µmol/l, is said to occur when glomerular fiteration rate is about 60ml/min and when end stage renal disease is reached, the prevalence of hyperhomocysteinemia will be between 85-100% [84]. Homocysteine have a strong correlation with glomerular filtration rate as reduced glomerular filtration rate increases circulating levels of homocysteine in chronic kidney disease patients. An experimental study demonstrated that hyperhomocysteinemia was decreased after kidney

transplantation consequently, it is documented that renal mechanisms inevitably are responsible for elevated homocysteine levels in chronic kidney disease patients [63].

The coagulation profile, assessed using D-dimer, was markedly abnormal in chronic kidney disease patients. The significantly elevated D-dimer levels recorded in this study suggests enhanced fibrinolytic activity or ongoing coagulation disturbances commonly associated with kidney dysfunction. D-dimer are proteins that indicates the existence of intravascular clot formation and reflects the early process of fibrinolytic activities thus playing a crucial role in the diagnosis of thromboembolism [25]. This finding conforms to other research works [85, 86]. D-dimer statistically significant negative correlation with platelet count indicates that higher D-dimer levels corresponds to lower platelet count. Basically, chronic kidney disease patients are known to have coagulation disorders with resulting thrombotic complication being the most common cause of mortality and posing a great difficulty in renal replacement therapy among chronic kidney disease patients [21].

The statistically significant systematic elevation of D-dimer across the different stages of chronic kidney disease recorded in this study shows that as kidney function declines, D-dimer levels increases. This result implies that chronic kidney disease may predispose individuals to hypercoagulable state, reflecting endothelial injury, inflammation and impaired renal clearance of fibrin degradation products as renal function declines [60]. This pro-thrombotic milieu contributes to the elevated cardiovascular risk observed in these patients and may be exacerbated by the uremic environment and inflammation [66].

Pearson's correlation showed that D-dimer had a significant negative correlation with estimated glomerular filtration rate. This conforms to other research works. Sheikh et al, (2021) in a cross sectional study on 98 patients attending a Nephrology clinic in Iran also reported that D-dimer negatively correlates to GFR and further stated that there were no evidence of thromboembolic events during their one year follow up. Another study by Xi, (2016) including 1784 participants published a mean D-dimer levels of 291.5 mg/l, 995.5 mg/l and 1901.5 mg/l in the patients with normal renal function, mild renal disease and moderate renal disease respectively and reported a significant relationship between D-dimer levels with estimated glomerular filtration rate. A work by Mohammrd and Khalil, (2016) had a contrary report as they found no significant relationship between D-dimer level and patients' glomerular filtration rate and age but reported a significant correlation between D-dimer levels and chronic kidney disease.

Based on the findings presented, homocysteine and D-dimer levels demonstrate a clear and statistically significant association with the progression of Chronic Kidney Disease. Homocysteine levels were significantly higher in end-stage renal disease patients compared to moderate and severe cases ($p = 0.008$), a pattern indicative of declining renal clearance and its role as a potential biomarker for cardiovascular risk in this population. Similarly, D-dimer levels was increased significantly with chronic kidney disease stage ($p = 0.028$), suggesting a state of heightened fibrinolytic activity and hypercoagulability in advanced renal disease, which aligns with research linking chronic kidney disease to endothelial dysfunction and coagulation abnormalities.

The correlation and regression analyses further elucidate the complex interplay between renal function, homocysteine and D-dimer. The significant positive correlation between homocysteine and creatinine ($r = 0.259$, $p = 0.009$) underscores their shared relationship with declining glomerular filtration. Furthermore,

the multinomial logistic regression identified urea and creatinine as powerful predictors of chronic kidney disease stage, with coagulation markers like D-dimer also contributing significantly to the model.

Collectively, these findings highlight the multifactorial nature of chronic kidney disease progression, where traditional markers of renal function, alongside homocysteine and coagulation indicator (D-dimer), provide a composite picture of disease severity. The regression model, which explained 64% of the variance in chronic kidney disease staging, demonstrates that a combination of biochemical and fibrinolytic parameters offers a robust framework for assessing disease progression. This supports the clinical utility of monitoring a panel of biomarkers, including homocysteine and D-dimer, to better stratify risk and guide management in chronic kidney disease patients, potentially allowing for earlier interventions to mitigate cardiovascular and thrombotic complications [80].

5.2 Conclusion

In conclusion, chronic kidney disease has a serious deleterious impact on the renal functions determined using electrolytes, urea and creatinine. The significant elevation of homocysteine and D-dimer in chronic kidney disease patients and across the different stages solidifies their role as key biomarkers for renal impairment. The findings strongly advocate for the integration of homocysteine and D-dimer testing into the routine clinical workup of chronic kidney disease patients to better stratify cardiovascular risk.

The demographic data reveals that chronic kidney disease predominantly affects middle-aged adults, with a notable male preponderance, and is strongly linked to modifiable risk factors, primarily hypertension and diabetes mellitus. A particularly critical finding is the alarmingly high proportion of patients (46%) presenting with end-stage renal disease, which underscores a significant failure in early detection and intervention strategies within the healthcare system. This late-stage presentation not only signifies a heavy disease burden but also points to an urgent need for improved public health initiatives focused on screening and early management.

5.3 Recommendations

Based on the findings of this study, the following recommendations are proposed to address the challenges identified in the diagnosis, management, and public health approach to chronic kidney disease.

1. Implement targeted screening programs for high-risk individuals, particularly those with hypertension, diabetes, or a combination of both. Routine checks should include not only serum electrolytes, urea and creatinine but also homocysteine and D-dimer levels, given its strong association with renal impairment.
2. Raise public awareness about the silent nature of early chronic kidney disease and promote regular health check-ups, especially for middle-aged and older adults, to reduce the high proportion of late-stage presentations.
3. Incorporate homocysteine testing into the standard diagnostic and monitoring panel for chronic kidney disease patients. Its role as a predictor of cardiovascular risk and renal progression can provide a more comprehensive assessment of patient prognosis.

4. Utilize D-dimer as supplementary tools to identify chronic kidney disease patients at high risk for thrombotic events and systemic inflammation, allowing for preemptive management strategies.
5. Establish integrated care models involving nephrologists, cardiologists, endocrinologists, and dietitians to manage the multifaceted complications of chronic kidney disease concurrently. This should focus on aggressive control of blood pressure and blood glucose,
6. Develop and disseminate educational materials for patients and their families focusing on the importance of medication adherence, dietary modifications (e.g., low-salt, low-protein, and potassium-controlled diets), and lifestyle changes to slow disease progression.
7. Enhance support systems for patients with advanced CKD, facilitating timely access to renal replacement therapies such as dialysis and transplantation, and providing psychosocial support to cope with the burden of a chronic disease.

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