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RELATIONSHIP BETWEEN HORMONE APELIN LEVEL AND SOME BIOCHEMICAL PARAMETERS IN IRAQI PATIENTS WITH KIDNEY DISEASE

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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By Ali Othman Obaid AL-ISAWI

January 2023

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality under our supervısıonsö ın partıal fulfıllment of the requırments for the degree of Master of Science

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ABSTRACT

RELATIONSHIP BETWEEN HORMONE APELIN LEVEL AND SOME BIOCHEMICAL PARAMETERS IN IRAQI PATIENTS WITH KIDNEY DISEASE

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Master of Science in Chemıstry

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January 2023

The kidney condition known as chronic kidney disease (CKD) is characterized by renal impairment, gradual loss of renal function. GFR is classified into 5 stages. Studies of apelin's function in Iraqi CKD patients were very few. Therefore, the aim of this thesis was to examine the relationship between the apelin hormone and many health-related variables and CKD in Iraqi patients. A case-control research was conducted from March to June 2022 at AL-Fallujah Teaching Hospital, AL-Anbar Province, Iraq, with 90 people (40 controls and 50 patients). Apelin levels in serum were significantly decreased in the CKD group than those in control group (268.98±117.36 vs 370.37±125.73 pg/mL: P= 0.002). The CKD group had high levels of urea nitrogen (101±37 mg/dL; p < .0001), creatinine (3.3±1.3 mg/dl; P< 0.0001), glucose (108±55 mg/dL; P = 0.0097), HbA1c (5.4±2.1%), K (4.9±0.82 mmol/L; P= 0.0222), and had low levels of albumin (3.0±0.7 g/dL; P= 0.042), Na (133.1±6.0 mmol/L; P< 0.001) as in comparision to the control group. According to the results of a correlation study, apelin was adversely connected with age, BMI, BUN, creatinine, K, and albumin (P< 0.05). While GFR and apelin had a positive correlation (P= 0.001). This research suggests that apelin may be a possible biomarker for assessing the severity and progression of CKD.

2023, 46 pages

Keywords: Chronic kidney disease, Apelin, BUN, Creatinine, Albumin, GFR

ÖZET

IRAK'TA BÖBREK HASTALIĞI OLAN HASTALARDA HORMON APELIN DÜZEYİ İLE BAZI BİYOKİMYASAL PARAMETRELER ARASINDAKİ İLİŞKİ

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Kronik böbrek hastalığı (KBH) olarak bilinen böbrek durumu, böbrek yetmezliği, kademeli böbrek fonksiyonu kaybı ile karakterizedir. GFR 5 aşamada sınıflandırılır. Irak KBH hastalarında apelinin işlevine ilişkin çalışmalar çok azdı. Bu nedenle bu araştırmanın amacı, Iraklı hastalarda apelin hormonu ile sağlıkla ilgili birçok değişken ve KBH arasındaki ilişkiyi incelemektir. Mart-Haziran 2022 tarihleri arasında Irak'ın AL-Anbar Eyaleti, AL-Fallujah Eğitim Hastanesinde 90 kişiyle (40 kontrol ve 50 hasta) bir vaka kontrol araştırması yapıldı. Serumdaki apelin seviyeleri, kontrol grubuyla karşılaştırıldığında KBH grubunda anlamlı olarak azaldı (268,98±117,36'ya karşı 370,37±125,73 pg/mL: P= 0,002). KBH grubunda üre nitrojen (101±37 mg/dL; P< 0.0001), kreatinin (3,3±1,3 mg/dL; P< 0,0001), glukoz (108±55 mg/dL; P= 0,0097), HbA1c (%5,4±2,1), K (4,9±0,82 mmol/L; P= 0,0222), düşük albümin (3,0±0,7 g/dL; P= 0,042), Na (133,1±6,0 mmol/L); P< 0.001) kontrol grubu ile karşılaştırıldığında. Bir korelasyon çalışması sonucuna göre apelin ile yaş, VKİ, BUN, kreatinin, K ve albümin arasında ters ilişki bulunmuştur (P< 0,05). GFR ile apelin arasında ise pozitif korelasyon vardı (P= 0.001). Bu araştırma, apelinin KBH'nin ciddiyetini ve ilerlemesini değerlendirmek için olası bir biyobelirteç olabileceğini düşündürmektedir.

2023, 46 sayfa

Anahtar Kelimeler: Kronik böbrek hastalığı, Apelin, BUN, Kreatinin, Albümin, GFR

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Ali Othman Obaid AL-ISAWI

Çankırı, 2023

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LIST OF SYMBOLS

-	Minus
%	Percent
/	Divide
+	Plus
<	Lesser than
>	Greater than
µL	Microliter
dL	Deciliter
h	Hour
IU	International Unit
K^+	Potassium Ion
L	Litre
m^2	Meter square
mg	Milligram
mIU	Milli-International Units
min	Minute
mL	Milliliter
mmol	Milimole
Na^+	Sodium Ion
nm	Nanometer
ºC	Degrees Celsius
OD	Optical density
pg	Picogram
rpm	Revolution per minute

LIST OF ABBREVIATIONS

ACE2	Angiotensin converting enzyme 2
AKI	Acute kidney injury
APJ	Apelin receptor
AT1	Angiotensin recptor
BMI	Body mass index
BUN	Blood urea nitrogen
cAMP	Cyclic adenosine monophosphate
CDCP	Centers for disease control and prevention
CKD	Chronic kidney disease
CKD-EPI	Chronic kidney disease epidemiology collaboration
DKD	Diabetic kidney disease
DM	Diabetes mellitus
eGFR	Evaluated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
ESRD	End-stage renal disease
GFR	Glomerular filtration rate
HbA1c	Hemoglobin A1c
HRP	Horseradish peroxidase
K	Potassium
KD	Kidney disease
KDOQI	Kidney disease outcomes – quality initiative
mRNA	Messenger ribonucleic acid
Na	Sodium
RAS	Renin-angiotensin system
SD	Standard Deviation,
SE	Standard Error,
TMP	Tetramethylbenzidine
US	United state

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1. INTRODUCTION

The CKD is a non-communicable disease characterized by various physiological abnormalities, declining renal function, and steadily declining GFR (Zeba *et al.* 2020). There are five stages of renal dysfunction in CKD, ranging from moderate impairment to complete renal failure (Forbes and Gallagher 2020). CKD is thought to affect 8% to 16% of people worldwide (at all stages), which could mean millions of deaths per year (Fatema *et al.* 2013). The end-stage of renal disease (ESRD) or even pre-onset mortality is much more likely to progress in patients with CKD at stages 3–5 (Das *et al.* 2019). Age, gender, cardiovascular disease, primary kidney disease, urinary tract infection, diabetes mellitus (DM), family history of kidney disease (KD) and nephrotoxins (nonsteroidal anti-inflammatory drugs, antibiotics) all contribute to CKD, recognized as a risk factor (Ghelichi-Ghojogh *et al.* 2022).

Apelin is a natural ligand of seven-transmembrane G protein-coupled-receptor APJ. Tissues of the kidneys, heart, lungs and tumor tissue are all homes for apelin and APJ. Studies have shown that the inner stripe of the outer medulla oblongata of the kidney has increased concentration of apelin-mRNA expression, which is important for the process of salt and water balance (Chapman *et al.* 2021; Huang *et al.* 2018). Apelin has important functions in the control of fluid balance and glomerular hemodynamics, according to mounting data, which suggests that apelin, has positive effects on KD physiology (Chapman *et al.* 2021). More studies also suggest that the apelin/APJ system has broad functions in the kidney. Overall, the apelin has diverse functions in KD and is a potential therapeutic target (Huang *et al.* 2018). As a result, we examine the role of apelin hormone in CKD patients.

1.1 Aim of research

1. To measurement the concentrartion of the apelin hormone in CKD Iraqi patients.

2. To investigate the association between apelin hormone and other markers (glucose, Hemoglobin A1c (HbA1c), urea nitrogen (BUN), creatinine, albumin, sodium (Na^+), and potassium (K^+) in CKD in Iraqis.

2. LITERATURE REVIEW

2.1 Apelin Hormone

Apelin is an adipokine known to be the endogenous ligand for the orphan receptor APJ. Apelin and APJ were highly expressed in variety of tissues, including kidney, heart, lung and blood vessels (Chapman *et al.* 2021). There were several biological uses for apelin and APJ system in renal disease. It was a key modulator of renal fibrosis, diabetic nephropathy, polycystic KD, renal ischemia and/or reperfusion injury, hemodialysis, and CKD. The apelin system is a novel component of the renin-angiotensin-system (RAS) and plays a vital role in vasodilation, decreased blood pressure, and increasing cardiac contractility (Huang *et al.* 2018). In addition, apelin regulates vasopressin release and fluid balance (Chapman *et al.* 2021).

2.1.1 Structure

Apelin was previously isolated from bovine stomach extract as an endogenous ligand for APJ (a putative receptor protein related to the angiotensin receptor AT1). The apelin gene on chromosome Xq 25–26 encodes human apelin. This peptide appears in several chemical forms with various biological functions and contains a 77-amino acid (AA) pre-proapelin precursor. Enzymatic hydrolysis of natural pre-proapelin and changed into active form results in apelin-13 (pre-proapelin 65-77), apelin-17 (pre-proapelin-61-77), and apelin-36 (pre-proapelin-42-77).and pyroglutamate (pyr-apelin-) (Figure 2.1) (Read *et al.* 2020, Kurowska *et al.* 2018).

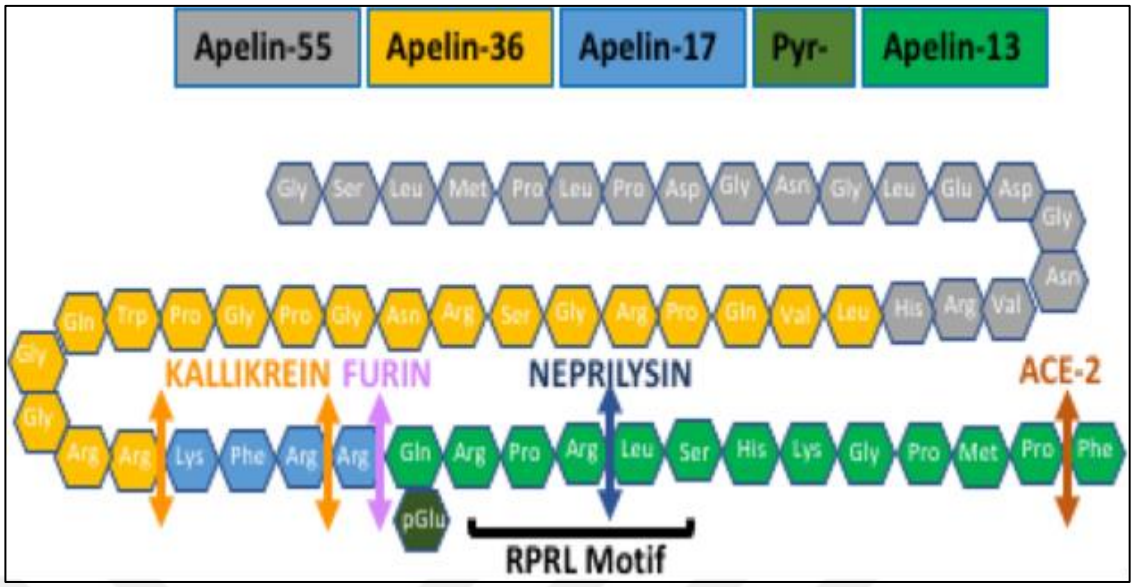

Figure 2.1 The hormone apelin in many forms (Janssens *et al.* 2022)

Both in vivo and in vitro experiments have been conducted with apelin-13 in several studies. This was because the short variant of apelin. Apelin-13 has much better potential biologically than the long variant (apelin-36) (Read *et al.* 2020).

The N-terminal section of apelin contains lot of hydrophobic AA, indicating that they constitute a secretory signal sequence, while C-terminus of apelin contains a sequence of 23-AA. It was preserved and necessary for biological function (Kurowska *et al.* 2018).

2.1.2 Receptor

The apelin gene, found on chromosome Xq 25–26, encodes the human apelin. The apelin gene also known as AGTRL1, APJR, and APJ encodes for this receptor. Due to the apelin receptor's sequence resemblance to the angiotensin II type 1 receptor, it was first classified as an orphan receptor; however, it was later deorphanized by the discovery of apelin in 1998. The peptides are degraded by the angiotensin converting enzyme 2 (ACE2), which in turn inhibits the agonistic actions of both apelin and angiotensin II (Kuba *et al.* 2019, Kurowska *et al.* 2018).

Apelin receptor interacts with the activation of several signaling mechanisms as a result of its varying affinities for distinct apelin forms and its cointeraction with multiple G such as Gα, Gβ, and Gγ proteins. Furthermore, the G protein-dependent pathways (calcium, ERK and cAMP) still showed unique activation patterns for the peptide ligands. Consequently, depending on the cell type, apelin receptors activate different G proteins and stimulate different intracellular signaling pathways. This may help explain how receptors mediate so many physiological effects (Kurowska *et al.* 2018).

2.1.3 Expression

In humans, the brain, stomach, lung, heart, spinal cord and kidney all have high levels of apelin mRNA expression, whereas other tissues exhibit lower levels (Chapman *et al.* 2021). Apelin protein is mostly found in vascular endothelial cells in the peripheral, such as those in the kidney and adrenal gland, as well as in the atria's endocardial endothelial cells and ventricles of the heart (Marsault *et al.* 2019). Adipocytes produce more apelin as they differentiate due to growth hormone, insulin, tumor necrosis factor, and other hormones that control apelin expression and production (Kurowska *et al.* 2018).

2.1.4 Function

The apelin signaling pathway controls energy metabolism, water and food intake, both blood flow and pressure and immune function in central and peripheral regulation of the CVD (Kurowska *et al.* 2018). Furthermore, in vivo studies, apelin has been noted to be a potent angiogenic factor that promotes migration, and proliferation of endothelial cells and blood vessel growth. Apelin could be important in controlling water balance.as apelin mRNA expression was found in brain regions important for fluid homeostasis control (O'Carroll *et al.* 2013).

Apelin mRNA is expressed in every kidney components and regulate renal tubular function. So expression of the apelin and APJ system could be crucial for renal

function. Studies suggest that apelin is also found in urinary catheters and may function as a micturition-enhancing peptide (Najafipour *et al.* 2012).

On the other hand, compared to healthy patients, plasma apelin levels are elevated in obese, obesity and hyperinsulinemia are associated, so this condition might be responsible for the increased expression of apelin. Lately, obese individuals have been shown to have the highest levels of apelin (Li and Chen 2012).

2.2 Chronic Kidney Disease (CKD)

It was described as a structural or functional abnormality of the kidney that has been present for more than three months. Estimated GFR (eGFR) is one of the primary categorization criteria (Forbes and Gallagher 2020). A verified formula should be used to determine the GFR from serum creatinine. The equation developed by the CKD-Epidemiology Collaboration equation (CKD-EPI) is often preferred. Laboratories commonly report GFR together with serum creatinine. Although the ideal limits for clinical diagnosis in older persons are debatable, a GFR consistently 60 mL/min/1.73 m^2 is deemed abnormal (ADAPPC 2022). It is determined by renal anotomical or functional abnormalities that have persisted for more than 3 months. An eGFR is one of the essential criteria for classification (Forbes and Gallagher 2020). Using proven methodologies, GFR should be determined from serum creatinine. The equation developed by the CKD-EPI is often preferred. Laboratories usually report serum creatinine and GFR. Although the optimal criteria for clinical diagnosis in the elderly are controversial, an eGFR consistently below 60 mL/min/1.73 m^2 was considered abnormal (ADAPPC 2022).

Globally, 336 million males and 417 million females were afflicted by CKD in 2016 (Bikbov *et al.* 2018). Additionally, the US Centers for Disease Control and Prevention (CDCP) estimates that 15% of adults in the US or 37 million people have CKD. Notably, 1 in 2 patients without dialysis who had severely poor renal function and 90% of adults with CKD were unaware that they have the disease. DM and high blood pressure were the main causes of CKD: One in three persons with DM and one in five

adults with hypertension may also develop CKD, according to the CDC. CKD is more prevalent in adults 65 years of age or older at a rate of 38% compared to those between 45 and 64 years of age (13%) or in people 18 to 44 years of age (7%) and it is somewhat more prevalent in women (15%) than in males (12%) according to the most recent CDC figures (Wilson *et al.* 2021).

2.2.1 Classification of CKD

The degree of renal function as determined by GFR determines the stage of CKD. Based on eGFR, the five stages of CKD are mentioned in Table 2.1 (Forbes and Gallagher 2020).

Table 2.1 CKD Classifications (Forbes and Gallagher 2020)

GFR category	range	description
1	90+	Normal kidney function
2	60-89	Mildly reduction kidney function related to normal range
3a 3b	45-59 30-44	Mildly to moderately reduction kidney function Moderately to severely reduction kidney function
4	15-29	Severely reduction kidney function
5	< 15	Very severely reduction kidney function (Kidney failure)

2.2.2 Diagnosis of CKD

This includes people who have abnormal urine sediment, albuminuria, electrolyte abnormalities, other abnormalities caused by tubular disorders, histology abnormalities, structural abnormalities found by imaging, history of renal transplantation, and GFR that are less than 60 mL/min/1.73 m^2 on two different occasions separated by at minimum of 90 days with or without renal impairment. To calculate eGFR, clinical laboratories should use the CKD-EPI. This should be done with caution in individuals with extremely high muscle mass, as loss of muscle mass overestimates GFR and high muscle mass underestimates (Forbes and Gallagher 2020).

2.2.3 Risk Factors of CKD

In order to focus screening programs on high-risk populations, it is important to finding the variables linked to a higher risk of developing CKD. The two main categories of risk factors for CKD are initiating variables that increase the likelihood of having CKD and permanent factors that increase the likelihood that CKD will progress to ESRD. A list of recognized risk factors for KD is shown in Table 2.2 (McClellan and Flanders 2003). According to analysis study, screening all patients over 60 is still cost-effective even when no other risk factors exist for CKD; however, screening low-risk individuals peopled under than 60 does not seem to be cost-effective (Snyder and Pendergraph 2005).

Table 2.2 CKD Risk Factors (McClellan and Flanders 2003)

Initiating factors	**Traditional factors or markers**
Age (years)	High protein intake
Gender	Proteinuria
Dyslipidaemia	Smoking
Family history of CKD	Africane-american race
Nephrotoxins	Obesity
Metabolic syndrome	Hypertension
Socio-economic status	Dyslipidaemia
Ethnicity	Nephrotoxins
High normal urinary albumin excretion	Anaemia
Primary kidney disease	Cardiovascular disease
Cardiovascular disease	
Urological disorders	
Diabetes mellitus	

2.2.4 Causes of CKD

The most typical cause of KD is diabetes. Between 40% and 60% of people who develop ESKD had diabetes. Patients with ESRD may also have other underlying illnesses, such as cystic KD (2 to 3 percent), glomerulonephritis (<10%), and

hypertension (15–30%). The remaining ESRD patients have unidentified etiology (Snyder and Pendergraph 2005). There are significant differences between countries, despite advances in understanding and diagnostic methods in many countries, the number of patients with ESRD for unknown reasons remains high (Evans and Taal 2011).

Causes of kidney disease (Evans and Taal 2011):

- Diabetes mellitus
- Drugs
- Genetic diseases
- Glomerulonephitis
- Infections
- Urological conditions

2.3 Actions of Apelin in the Kidney

Despite the fact that the kidneys express apelin at high amounts.Studies on apelin's function in human renal physiology were sparse. Animal studies show that apelin directly affects the kidneys, affecting renal hemodynamics and fluid balance. RAS tightly controls glomerular blood flow and also regulates glomerular perfusion and GFR. Furthermore, the RAS and apelin systems communicate with each other (Figure 2.2). Apelin reverses the vasoconstriction that Ang II-induced efferent glomerular arterioles of rats, causing a dramatic decline in intracellular calcium levels.. Apelin's vasodilatory effects require healthy endothelium and NO synthesis. Apelin-induced vasodilation may promote diuresis by increasing renal medullary blood flow as efferent arterioles supply the vascular rectum (Figure 2.2) (Chapman *et al.* 2021).

Apelin helps maintain fluid homeostasis by counteracting vasopressin's effects, and there is intersystem communication as a result. These findings are supported by a clinical study in healthy individuals in which a parallel reciprocal increases in apelin

levels occurred concurrently with changes in plasma osmolality, and vasopressin. Central or peripheral injection of apelin peptide causes animals to produce more urine.

The signaling mechanism in rat collecting duct is mediated by interaction with apelin and the vasopressin receptor, apelin prevents vasopressin from inducing water reabsorption. Furthermore, apelin does not affect the kidney's ability to process sodium or potassium, as it acts directly on the kidney tubules to cause dose-dependent aquaresis (Hus-Citharel *et al.* 2014).

Studies performed in vitro and in vivo indicate that suppression of aquaporin 2 channel insertion into the apical cellular membranes of collecting ducts is responsible for this aquaresis (Figure 2.2) (Boulkeroua *et al.* 2019, Hus-Citharel *et al.* 2014).

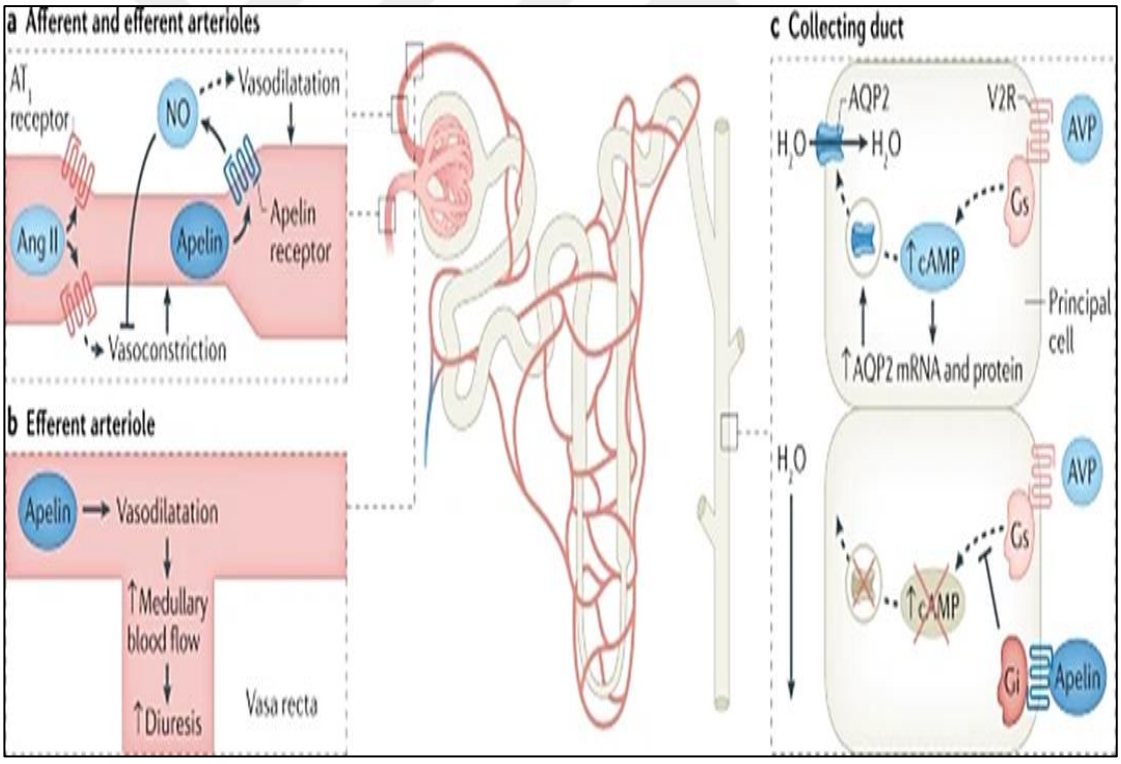

Figure 2.2 The actions of apelin in the kidney (Chapman *et al.* 2021)

Apelin has antiinflammatory and antifibrotic properties that protect against various disorders. It might also be crucial in certain KD.More than 50% of patients admitted to

critical care have acute kidney injury (AKI), affecting 8-17% of hospital admissions (Sawhney *et al.* 2017). Furthermore, the incidence of CKD, renal failure, and mortality are all strongly associated with AKI. Studies using AKI show that the apelin system is initially upregulated before being downregulated as injury progresses. This dynamic shift resembles to what occurs in heart failure. In renal ischemia/reperfusion, a frequently used model of AKI, apelin therapy has a direct protective effect supported by both in vitro and in vivo evidence (Chapman *et al.* 2021; Gholampour *et al.* 2020).

2.4 Relation of Apelin Levels with CKD

The CKD was one of the main global causes of morbidity and death, has a separate relationship with cardiovascular disease. Blocking RAS is a cornerstone of CKD treatment because it lowers blood pressure, reduces proteinuria, and delays loss of renal function. Despite this therapy, many patients develop renal failure requiring dialysis or renal transplantation and/or die of cardiovascular disease. A growing list of diseases may benefit from targeting the apelin system as a possible treatment (Chapman *et al.* 2021).

Numerous in vitro and in vivo researches have studied how the apelin mechanism affects kidney patholog. Apelin has anti-inflammatory and anti-fibrotic properties that protect against various disorders. It may also play a significant role in some KD (De-Mota *et al.* 2004).

The apelin system interacts with RAS to modulate glomerular perfusion, GFR, and other factors, to tightly regulate GFR, Both the efferent and the afferent arterioles are constricted by Ang II, by increasing intracellular calcium levels, with a focus on efferent arterioles. Apelin helps maintain fluid balance by counteracting the effects of vasopressin and causing systemic crosstalk (De-Mota *et al.* 2004). Clinical investigations in healthy individuals complement these findings that variations in plasma osmolality are accompanied by corresponding variations in the levels of vasopressin and apelin (Azizi *et al.* 2008). Due of Ang II's impact on the AT1 receptor, the RAS promotes kidney fibrosis, and blocking the system is advantageous in CKD

(Wang *et al.* 2017). Additionally, apelin promotes dose-dependent aquaresis by acting directly on the renal tubules without affecting renal potassium or sodium handling (Hus-Citharel *et al.* 2014). Studies performed in vitro and in vivo point to the fact that the insertion of aquaporin 2 channels into the apical plasma membrane of collecting ducts is inhibited as a cause of this aquaresis (Boulkeroua *et al.* 2019).

There is still controversy regarding the circulation of apelin peptides in CKD patients. One study found that dialysis-dependent CKD patients had low plasma apelin concentrations, which were associated with cardiac function (Małyszko *et al.* 2006). Another study discovered no variation in apelin 36 levels in these individuals, although levels of apelin 12 were greater than in healthy people group (Leal *et al.* 2012). A substantial reduction in apelin was found in renal transplant recipients with coronary heart disease and was independently associated with indices of endothelial dysfunction or inflammation (Malyszko *et al.* 2008). In support, a recent study examined the relationship between apelin levels and indicators of renal and cardiovascular dysfunction and found that plasma apelin was a greater level in hemodialysis patients comparable to healthy controls (Doğan *et al.* 2018a).

2.5 Relation of Electrolyte Levels with CKD

CKD patients may have electrolyte problems in 3% to 11% of cases (Inker *et al.* 2019). Dietary restrictions and supplement prescriptions are often part of the initial treatment plan. Primary care physicians should advise a low-potassium diet for hyperkalemia and a low-phosphate diet for hyperphosphatemia. Because studies have shown that chronic metabolic acidosis is linked with a higher incidence of CKD, chronic blood bicarbonate levels below 22 mmol/L are recommended. In patients, oral bicarbonate therapy should be taken into consideration (Inker *et al.* 2014).

According to studies examining the care of patients with CKD, patients should also be aware of the consequences of CKD such as hyperkalemia, metabolic acidosis, anemia, and other metabolic abnormalities (Chen *et al.* 2019).

In the study by Sofue *et al.* 2020, they showed the prevalence of electrolyte disorders in 35,508 patients in Japan with CKD. Advancement in the eGFR-5 category was associated with significant increase in mean blood K^+ and phosphate concentrations and a decrease in average serum chloride and corrected calcium concentrations. However, patients with eGFR categories of ≥5 and <10 mL/min/1.73 m^2 had higher mean blood chloride and adjusted calcium concentrations compared with those with eGFR categories of ≥10 and <15 mL/min/1.73 m^2. As the G category progressed, so did the percentage of patients with low serum Na^+ levels. Also the proportion of individuals with both low and high serum-adjusted calcium levels increased as the G category progressed. Furthermore, it is expected that the prevalence of G-category development-related electrolyte disturbances may alter over time when new medications are introduced. As a result, individuals with CKD should be candidates for long-term studies (Sofue *et al.* 2020).

2.6 Relation of Diabetes Mellitus with CKD

In diabetes, the kidney may be the most important site of microvascular damage. Most people with diabetes experience kidney disease due to diseases such as hypertension, age-related nephron loss, and/or other comorbidities.

CKD prevalence and severity can be used to identify individuals who are more likely to have poor health outcomes and die prematurely. As a result, one of the main goals of holistic patient care today is to prevent and manage CKD in diabetic patients. Controlling blood glucose levels and blood pressure are all part of intensive care for patients with diabetes. These measures will reduce the incidence of diabetic kidney disease and cease progression. Indeed, improved diabetes management has contributed significantly to the significant reduction in the incidence of diabetic kidney disease (DKD) and improved patient outcomes over the last three decades. However, innovative therapeutic approaches are still needed to prevent arrest, treat and reverse DKD (Thomas *et al.* 2015).

Optimal diabetes management is also crucial. Most of the recommendations call for a target HbA1c of less than 7.0%. First, glycemic control may halt the progression of CKD. Second, it may be necessary to change the dosage of oral hypoglycemic agents. In general, drugs like glyburide that are largely excreted by the kidneys should be avoided, while drugs that are Processed by the liver and partially eliminated by the kidneys (metformin, sodium-glucose cotransporter-2 inhibitors and some dipeptidyl peptidase 4) may need to be dose reduced or discontinued, especially if the eGFR values were below 30 mL/min/1.73 m^2 (Chen, *et al.* 2019). Therefore, one of the main goals of holistic patient care is the prevention and management of CKD in patients with DM.

3. MATERIALS AND METHODS

3.1 Research Design

A patient was considered to have CKD if renal functional problems persist over a period of 3 months. Anyone with an eGFR less than 60 mL/min/1.73 m^2 was considered to have CKD. CKD should be staged according to the criteria of Kidney-Disease-Outcomes-Quality-Initiative (KDOQI) and should take into account the percentile distribution of eGFR by age and gender. A five-step CKD classification scheme based on GFR level was created by the national kidney foundation (Levey *et al.* 2002).

3.1.1 Patients

Fifty CKD patients were with an eGFR values less than 60 mL/min/1.73 m^2. The subjects were 31 males and 19 females, with a mean and standard devition age of 53.8±10.6 years, ranging from 31 to 72 years old. Patients with CKD stages 3-5 were usually considered to ranges from moderate to severity KD. There were two phases of renal impairment in stage 3-a; eGFR of 45-59 mL/min/1.73 m^2 and 3-b; eGFR of 30-44 mL/min/1.73 m^2. Wheres eGFR for stage 4 was between 15 and 29 mL/min/1.73 m^2, and stage 5 was an eGFR values less than 15 mL/min/1.73 m^2 (Levey *et al.* 2009 and Forbes and Gallagher 2020).

3.1.2 Healthy Subjects (Control)

A random sample of 40 apparently healthy individuals (24 males and 16 females) with average age 44.6±8.2 years, between the ages of 30 and 63 years, was selected as controls. Persons with normal GFR (GFR > 90) in the calculation of eGFR as well as individuls who indicated no history of CKD were included as controls.

3.2 Sample Collection

An overnight fast was followed by the drawing of blood samples from a peripheral vein. Centrifuging the samples at 5000 rpm for 10 min after being at 25°C for about 30-45 min to separate the serum. To preserve it for analysis, it was promptly frozen at -70 °C.

Serum apelin concentration was determined using an immune-enzymatic assay with a commercial ELISA kit for standard human apelin (provided by Elabscience Biotechnology Co., Ltd.) provided by the manufacturer. The Clinical Chemistry Analyzer used a colorimetric method to test plasma glucose, BUN, creatinine, albumin, Na^+, and K^+ levels (Abbott launches ARCHITECT c4000). Using a colorimetric method (AFIAS-10), an automated clinical chemistry system designed for use in clinical laboratories assessed HbA1c.

Patient age, gender, weight, and height were recorded based on the patient's chart. Between March and June 2022, all patients and controls were seen at AL Fallujah Teaching Hospital, Al Anbar Province, Iraq.

Inclusion criteria: Iraqis had to be over 20 years of age and had a definite CKD diagnosis by a nephrologist.

Exclusion criteria: Subjects were not included in the study if they had cognitive impairment, had a kidney transplant, or were seriously ill. Along with whether they were pregnant, took steroids, had autoimmune conditions, cancerous tumors, or liver illness.

3.3 Method

3.3.1 Measurement of Serum Apelin Levels

Apelin levels were measured in blood by using a commercial ELISA kit from Elabscience, Wuhan, China. The detection range is 62.5 to 4000 pg/mL with inter- and intra-test CV <10%.

Procedure Guidance: This ELISA kit uses competitive-ELISA technology. The microtiter plates in this kit were precoated with apelin. Apelin in the serum sample or standard competes with a fixed amount of apelin on the solid support for apelin-specific sites on the biotinylated-detection-Ab during the process. Excess conjugate and unbound samples or standards were removed from the plate and each microplate well was loaded with avidin conjugated to HRP and incubated. A substrate of TMB solution was then added to each well. A sulfuric acid solution was used to stop the enzyme-substrate reaction and the color change was detected spectrophotometrically at 450 nm±2 nm wavelength.

The OD of the samples may then be compared to the standard curve to calculated the concentration of APLN in the test samples.

Sensitivity: The limit of detection for human APLN was 37.5 pg/mL. The sensitivity of this assay was referred to as the lowest protein levels that can be distinguished from zero.

Detection Range: 62.5-4000 pg/mL.

Repeatability: Coefficient of variation was less than 10%.

Test procedures: All samples and reagents should be at room temperature before use. Material should be thawed before testing and then centrifuged again. All reagents

should be mixed properly by gentle swirling before pipetting. Do not lather. We recommend running all standards and samples in duplicate.

• Sample and biotinylated-detection-Ab: Add 50 μL of blank, standard or sample per well. The reference standard and sample diluent is applied to the blank well. 50 μL of the biotinylated-detection-Ab working solution should be added to each well right away. Use the plate sealer we gave to cover. Tap the plate lightly to achieve even mixing. At 37 °C, incubate for 45 minutes. (Solutions are applied to the bottom of the micro ELISA plate, being careful not to foam or contact the inner wall).

• Wash: Aspirate each well and wash three to four times. Using wash buffer (350 μL) to wash each well by using a squirt bottle, manifold dispenser, multichannel pipette, or automated washer. Liquid must be completely removed at each stage for it to work well. After the last wash, aspirate or decant any remaining wash buffer. Invert the plate and dab it with a thick piece of clean absorbent paper.

• HRP Conjugate: Fill each well with 100 μL of the HRP conjugate working solution. Add a fresh plate sealer on top. At 37 °C, incubate for 30 minutes.

• Wash: Perform the aspiration/wash procedure as described in step 4 five more times.

• Substrate: Fill each well with 90 μL of the substrate solution. Add a fresh plate sealer on top. At 37°C, incubate for approximately 15 minutes. Shield against light. Depending on the actual color shift, the reaction time may be cut or prolonged, but not beyond 30 minutes. When an apparent gradient appears in conventional wells, the reaction can be stopped.

• Stop solution: Fill any well with 50 μL of the stop solution. Immediately becomes yellow in color. Stop solution should be added in the same sequence as substrate solution.

• Optical density (OD): Measure the OD of each well simultaneously using a microplate reader set at 450 nm. Set the test settings, warm the equipment, and open the microplate reader beforehand.

• After the experiment, all unused chemicals reagents should be refrigerated at the proper storage temperature until the expiration date.

Calculating Results: Average each standard's and each sample's duplicate readings. By comparing the concentration on the X-axis to the OD average value of each standard on the Y-axis, the points on the graph can be connected with a curve of best fit to create a standard curve. We recommend using dedicated software such as Curve Expert 1.3 to perform this calculation.

Use the OD values and concentrations of standard samples to create the optimal standard curve equation in the software interface. Enter the OD value of the sample and the program will determine its concentration. If the sample was diluted, it was necessary to multiply the concentration determined from the standard curve by the dilution factor.

3.3.2 Measurement of Serum Creatinine Levels

Abbott GmbH and Co. Kit (Germany) No. 3L81-23 from Japan was used for assessment of serum creatinine levels by the ARCHITECT System Operation Manual.

Procedure Guidance: A creatinine-picric acid complex was created when sample creatinine interacts with picric acid at alkaline pH.

The amount of creatinine present in the sample directly correlates with the rate at which the sample's absorbance at 500 nm increases as a result of the development of this complex.

Detection range according to Table 3.1:

Table 3.1 Range of serum creatinine levels

Serum or Plasma	Range (mg/dL)	Range (µmol/L)
Teenage	0.5 to 1.0	0 44 to 88
Male adult	0.7 to 1.3	62 to 115
Female adult	0.6 to 1.1	53 to 97

Precision: The Creatinine serum assay has an imprecision of ≤ 6%. Total CV at values greater than 1.0 mg/dL and SD of < 0.1 mg/dL at concentrations lower than 1.0 mg/dL.

3.3.3 Measurement of Serum Urea Nitrogen (BUN) Levels

Abbott GmbH and Co. Kit (Germany) No. 7D75-22 from USA was used for assessment of BUN levels by the ARCHITECT System Operation Manual.

Procedure Guidance: The BUN assay was a revision of the complete enzymatic process. The first reaction, the test was performed as a kinetic assay and the rate of initiation of the reaction was linear for a short time. Urease hydrolyzes the urea in the test sample to produce ammonia and carbon dioxide. In the second process, glutamate dehydrogenase oxidizes reduced nicotinamide adenine dinucleotide (NADH) to nicotinamide adenine dinucleotide (NAD), converting ammonia and a-ketoglutarate to glutamate and water. Two moles of NADH are oxidized for each mole of urea present. The initial rate of 340 nm absorbance decline was related to the initial urea levels of the test sample.

Detection range: According to Table 3.2

Table 3.2 Range of blood urea nitrogen (BUN) levels

Serum/Plasma	Age (years)	BUN (mg/dL)	BUN (mmol/L)
Male	< 50	8.9 - 20.6	3.2 - 7.4
	> 50	8.4 - 25.7	3.0 - 9.2
Female	< 50	7.0 - 18.7	2.5 - 6.7
	> 50	9.8 - 20.1	3.5 - 7.2

3.3.4 Measurement of Electrolytes (Na^+ and K^+) Levels

Electrolyte levels in serum were detected using an automated analyzer (Fujifilm-Dri-Chem NX500i),

Procedure Guidance: Drop 50 mL of standard solution and 50 mL of whole blood, plasma, or serum onto the reference side and sample side of FUJI DRICHEM SLIDE Na-K-CI at the same time. After deposition, a stable ionic bond was formed as the references fluid and specimen spread along the dispersion device and approach each other on a unique threaded bridge. The two half-cells generate a differential voltage with respect to each other. The potential difference is proportional to the logarithm of the ion concentration ratio for each liquid. The FUJI DRI - CHEM ANALYZER incubates slides for a given time while measuring the potential difference between the reference and sample. The analyzer is then preloaded with potential difference values. A standard curve was used to convert the concentration of each electrolyte.

Detection range: Na^+: 136-149 mmol/L, K^+: 3.9- 5.0 mmol/L

3.3.5 Measurement of Albumin Levels

Abbott GmbH and Co. Kit (Germany) No. 61038UN21 from USA was used for assessment of albumin levels by the ARCHITECT System Operation Manual.

Procedure Guidance: A biochromatic digital endpoint approach is utilized to measure albumin concentration. This conjugate is formed by the reaction of albumin with the bromocresol purple reagent. The system tracks absorbance fluctuations at 600 nm. The quantity of albumin in the test was directly proportional to the change in absorbance.

Detection range 3.5- 5.2 g/dL

3.3.6 Measurement of Serum Glucose Levels

Abbott GmbH and Co. Kit (Germany) No. 3L82-22 from Canada was used for assessment of glucose levels by the ARCHITECT System Operation Manual.

Procedure Guidance: Hexokinase phosphorylates glucose in the existence of adenosine triphosphate and magnesium ions to form glucose-6-phosphate (G-6-P) and adenosine diphosphate. Specifically, glucose-6-phosphate dehydrogenase (G-6 PDH) reduces NAD to NADH while oxidizing 6-phosphat to 6-phosphogluconate. One micromole of NADH was produced for each micromole of glucose ingested. The resulting NADH can be confirmed spectrophotometrically as an increase in absorbance and absorbs light at 340 nm. Methodology: Hexokinase/G-6-PDH as method.

Detection range: 70 – 99 mg/dL

3.3.7 Measurement of HbA1c % Levels

The Boditech Med Inc. kit, code SMFP-28, from Korea, was used to assess the amount of Glycosylated Hemoglobin HbA1c % by AFIAS.

Procedure Guidance: A sandwich immunodetection technique was used in the test, in which detection antibodies in a buffer bind to antigens in the test sample to form antigen-Ab complexes. This migrates onto the nitrocellulose matrix and was captured by other immobilized antibodies on the test strip. The AFIAS test device gives the amount of glycated hemoglobin as a percentage of total hemoglobin in the blood. The more antigens in the test sample, the more antigen-Ab complexes created and the stronger the fluorescence signals of the detection Ab.

Detection range: 4% to 5.4%.

3.3.8 Measurement of eGFR Levels

The recently created CKD-EPI equation, which eGFR, has been suggested to be more precise and accurate (Levey *et al.* 2009). We measured eGFR, a marker of disease severity, in CKD patients' group with eGFR less than 60 mL/min/1.73 m^2 and compared them to controls subjects with an eGFR more than 90 mL/min/1.73 m^2.

3.4 Statistics Interpretation

Findings were presented as mean±SD or percentage change, depending on what was applied. Student's t-test and Spearman's correlation coefficient were also utilized to analyze differences between means and correlations between variables. P value less than 0.05 were used to indicate statistical significance. Statistical analysis was performed using software called SPSS vertion 11.0.

4. RESULTS AND DISCUSSION

4.1 The Clinical Characteristics of CKD

Table 4.1 provides clinical information on CKD patients as well as other recognized risk factors.

Table 4.1 Clinical data of the CKD patients

Age (years) Mean±SD		53.80±10.68
Gender (Female/male)		ratio 19/31
BMI (k/m^2) Mean±SD		26.40±2.98
Co-morbidities	Heart disease (n/%)	22 (44%)
	Diabetes mellitus (n/%)	10 (20%)
	Hyperlipidemia (n/%)	7 (14%)
	Hypertension (n/%)	12 (24%)
CKD Stage prevalence	3 (n/%)	12 (24%)
	4 (n/%)	22 (44%)
	5 (n/%)	16 (32%)

4.2 The Result of Apelin

Table 4.2, apelin levels in the CKD group group (268.98±117.36 pg/mL) were lower than in the control group (370.37±125.73 pg/mL) with a P-value of 0.002.

Table 4.2 The results of apelin in CKD patients and control

Parameters / **Groups**	**Mean±SD**	
	CKD (n=50)	Control (n=40)
Apelin (pg/mL)	268.98±117.36	370.37±125.73
Difference	-101.4150	
SD	121.9758	
SE	25.8750	
95% CI	-152.8361 to -49.9939	
t- Test	-3.919	
DF	88	
P value	*0* .002	

CI: Confidence interval for the difference between the means, t- Test: Test statistic t, DF: Freedom degrees.

These finding agree with (Doğan *et al.* 2018b) found peritoneal dialysis patients often have reduced apelin-13 levels, which may contribute to the development of inflammation, atherosclerosis, and dysfunctional endothelial cells. Apelin-13 levels much lower than in normal controls may be caused by the fact that patients with high peritoneal permeability have lower levels of apelin-13, supporting apelin-13 clearance through large peritoneal pores. According to Dorgan *et al.* (2018b), decreased apelin levels are also associated with indicators of inflammation and atherosclerotic processes. According to (El-Shehaby *et al.* 2010), Patients with ESRD had considerably reduced serum apelin assessed, correlated significantly with echocardiographic results, and contribute to the etiology of coronary heart disease in persons with CKD.

Apelin mRNA was expressed in all components of the kidney and may regulate renal tubular function. Studies suggest that apelin was also found in urinary catheters and may function as a micturition-enhancing peptide in urinary catheters (Najafipour *et al.* 2012).

4.3 The Result of eGFR

Table 4.3, eGFR levels in CKD patients were significantly lower at 22.90±10.0 vs 110.0±21.29 mL/min/1.73m^2 in contrast to controls with a P value < 0.0001.

Table 4.3 The results of eGFR in CKD patients and control

Parameters / **Groups**	**Mean±SD**	
	CKD (n=50)	Control (n=40)
eGFR (mL/min/1.73m^2)	22.9±10.0	110.0±21.29
Difference	87.1000	
SD	11.6933	
SE	2.4805	
95% CI	82.1705 to 92.0295	
t- Test	35.114	
DF	88	
P value	< 0.0001	

CI: Confidence interval for the difference between the means, t- Test: Test statistic t, DF: Freedom degrees.

Estimated GFR and apelin levels are positively correlated (Figure 4.1) (Silva *et al.* 2013). Prominent indicators of renal fibrosis are extracellular matrix proteins that enhance glomerular and renal interstitial abnormalities and increase the extent of renal damage. It was demonstrated by (Sun *et al.* 2016) the APJ system may reduce extracellular matrix deposition and ameliorate fibrosis of the renal interstices. Consequently, the APJ (apelin) system may have a renoprotective effect; this can be used to slow the development of CKD.

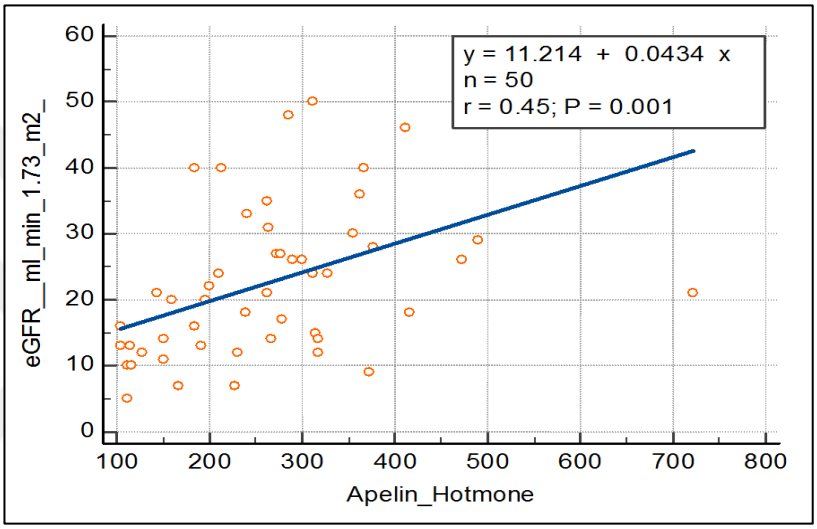

Figure 4.1 Correlation between apelin and eGFR in patients with CKD

4.4 The Result of Age

Table 4.4 and Figure 4.2 compare the average ages of the two groups 53.8±10.68 years in CKD patients and 44.6±8.26 years in controls. There was a significant difference with a P-value of 0.0001.

These results were similar to (Karadag *et al.* 2014) shown that apelin concentration were inversely associated to age and left ventricular diameter in peritoneal dialysis patients.

According to the Wilson *et al.* (2021), The CKD was slightly higher prevalent in female (15%) in comparison to males (12%) and was more prevalent among those aged 65 and older (38%) than to those aged between 45 and 64 (13%), and between 18 and 44 years old (7%).

Table 4.4 The results of age in CKD patients and control

Parameters / **Groups**	**Mean±SD**	
	CKD (n=50)	Control (n=40)
Age (years)	53.80±10.68	44.60±8.267
Difference	9.2000	
SD	9.6893	
SE	2.0554	
95% CI	5.1153 to 13.2847	
t- Test	4.476	
DF	88	
P value	< 0.0001	

CI: Confidence interval for the difference between the means, t- Test: Test statistic t, DF: Freedom degrees.

Age and apelin were negatively associated in a correlation study (P-value 0.01). These results were agreed with (Doğan *et al.* 2018b).

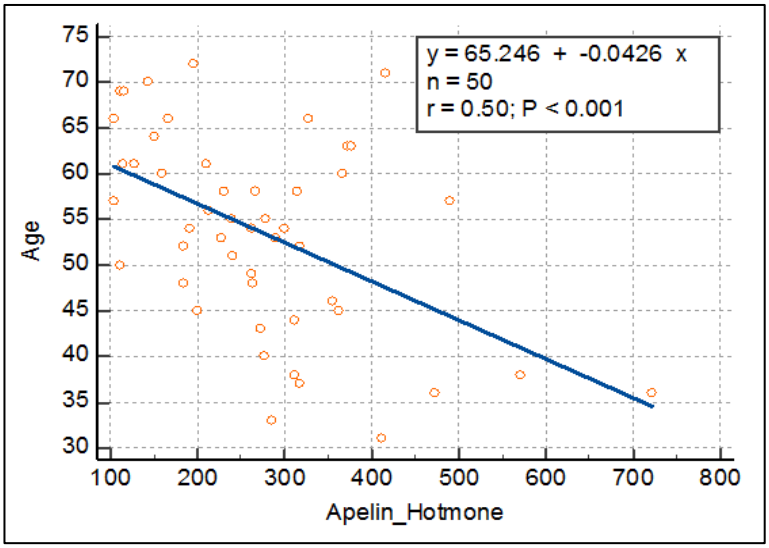

Figure 4.2 Correlation between apelin and age in patients with CKD

4.5 The Result of BMI

The difference in BMI between the two groups is shown in Table 4.5 and Figure 4.3. Mean BMI in CKD patients was 26.4±2.98 kg/m^2 and 27.28±3.05 kg/m^2 in controls. No change seen (P-value=0.161).

Table 4.5 The results of BMI in CKD patients and control

Parameters / **Groups**	**Mean±SD**	
	CKD (n=50)	Control (n=40)
BMI (kg/m^2)	26.40±2.98	27.28±3.05
Difference	-0.8875	
SD	2.9635	
SE	0.6287	
95% CI	-2.1368 to 0.3618	
t- Test	-1.412	
DF	88	
P value	0.161	

CI: Confidence interval for the difference between the means, t- Test: Test statistic t, DF: Freedom degrees.

A correlation study found that apelin and BMI were inversely associated (P-value 0.01). These findings were similar to (Doğan *et al.* 2018b).

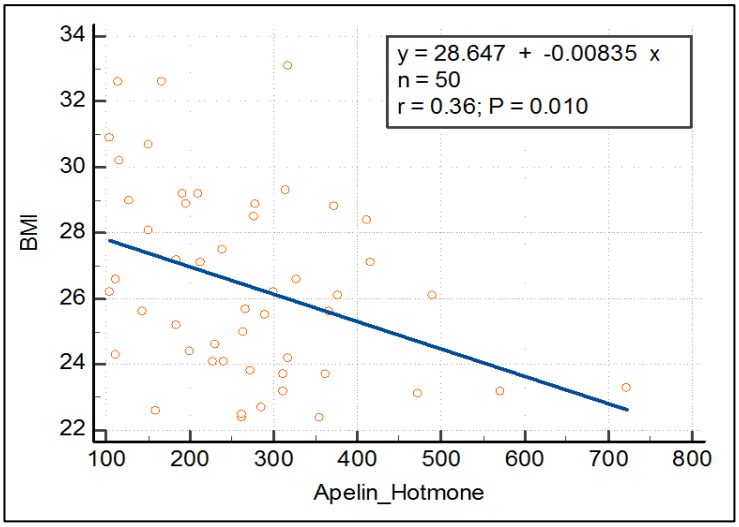

Figure 4.3 Correlation between apelin and BMI in patients with CKD

4.6 The Result of Urea Nitrogen (BUN) and Creatinine Levels

Tables 4.6 and 4.7, levels of BUN and creatinine in CKD patients were significantly higher compared to healthy controls (101.26±37.51 vs 19.57±6.48 mg/dL and 3.32± 1.37 vs. 0.876±0.12 mg/dL; P<0.0001, respectively). These results were agreed with Doğan *et al.* (2018b) study.

Table 4.6 The results of urea nitrogen levels in CKD patients and control

Parameters / **Groups**	**Mean±SD**	
	CKD (n=50)	Control (n=40)
Urea nitrogen (mg/dL)	101.26±37.51	19.57±6.48
Difference	-81.6850	
SD	28.3235	
SE	6.0083	
95% CI	-93.6253 to -69.7447	
t- Test	-13.595	
DF	88	
P value	< 0.0001	
CI: Confidence interval for the difference between the means, t- Test: Test statistic t, DF: Freedom degrees.		

Table 4.7 The results of creatinine in CKD patients and control

Parameters / **Groups**	**Mean±SD**	
	CKD (n=50)	Control (n=40)
Creatinine (mg/dL)	3.32±1.37	0.87±0.12
Difference	-2.5208	
SD	1.0286	
SE	0.2182	
95% CI	-2.9545 to -2.0872	
t- Test	-11.553	
DF	88	
P value	< 0.0001	
CI: Confidence interval for the difference between the means, t- Test: Test statistic t, DF: Freedom degrees.		

Results show an increase in BUN and creatinine, as BUN is affected by protein uptake, catabolism, and tubular urea reabsorption. Serum creatinine was freely filtered in the glomerulus and traverses through tubular secretions, rather than being reabsorbed.

Because of this flow dependence of urea reabsorption, more urea was reabsorbed with less urine flow (Schrier 2008).

A correlation study found a negative association between BUN and creatinine concentrations with apelin. Figure 4.4 and 4.5 these findings were agreed with results by Doğan *et al. (*2018b).

The activation of the apelin mechanism may be crucial in the kidney, according to Najafipour *et al.* (2012), apelin protein was expressed in all renal components and may control how well the renal tubule functions. Apelin was also found in urinalysis catheter, which suggests it may function as a pro-drainage there.

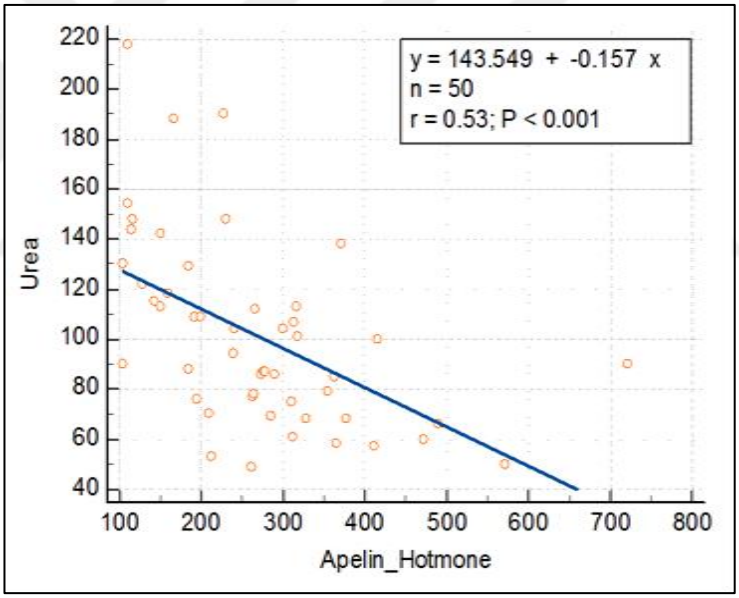

Figure 4.4 Correlation between apelin with urea in patients with CKD

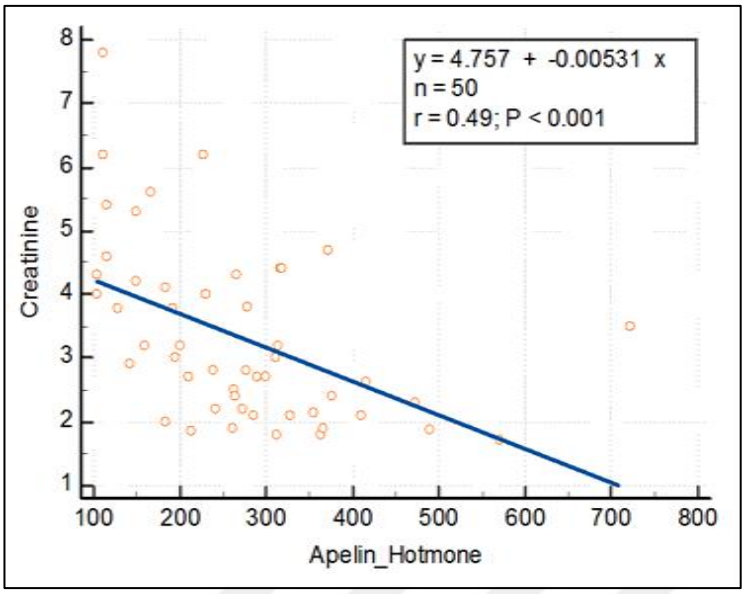

Figure 4.5 Correlation between apelin with Creatinine in patients with CKD

4.7 The Result Serum Electrolyte Levels

As shown in Tables 4.8 and 4.9, Na^+ levels in CKD patients were lower and K^+ levels was higher than in controls: 133.12±6.04 mmol/L vs. 140.63±4.10 mmol/L and 4.91±0.82 mmol/L vs 4.57±0.49 mmol/L (Figure 4.6 and 4.7).

Table 4.8 The results of Na^+ in CKD patients and control

Parameters / **Groups**	**Mean±SD**	
	CKD (n=50)	Control (n=40)
Na^+ (mmol/L)	133.12±6.04	140.63±4.10
Difference	7.2050	
SD	5.27	
SE	1.114	
95% CI	4.9824 to 9.4276	
t- Test	6.442	
DF	00	
P value	<0.001	

CI: Confidence interval for the difference between the means, t- Test: Test statistic t, DF: Freedom degrees.

Studies have shown that the rat kidney's whole nephron structure expresses apelin/APJ. mRNA (Sagiroglu *et al.* 2012). Human collecting ducts have also been shown to exhibit apelin immunoreactivity (De-Falco *et al.* 2002). Furthermore, renal vascular endothelial cells are a major host for apelin expression (Kleinz and Davenport, 2004). Apelin receptors expression was most highly expressed in the collecting ducts, and increasing corticomedullary gradient (Hus-Citharel *et al.* 2008). Also, in the central stripe of the outside medulla of kidney, apelin was closely associated with the water-sodium balance (Khan *et al.* 2010).

Table 4.9 The results of K^+ in CKD patients and control

Parameters / **Groups**	**Mean±SD**	
	CKD (n=50)	Control (n=40)
K^+ (mmol/L)	4.91±0.82	4.57±0.49
Difference	-0.3448	
SD	0.6982	
SE	0.1481	
95% CI	-0.6391 to -0.05047	
t- Test	-2.328	
DF	88	
P value	0.0222	

CI: Confidence interval for the difference between the means, t- Test: Test statistic t, DF: Freedom degrees.

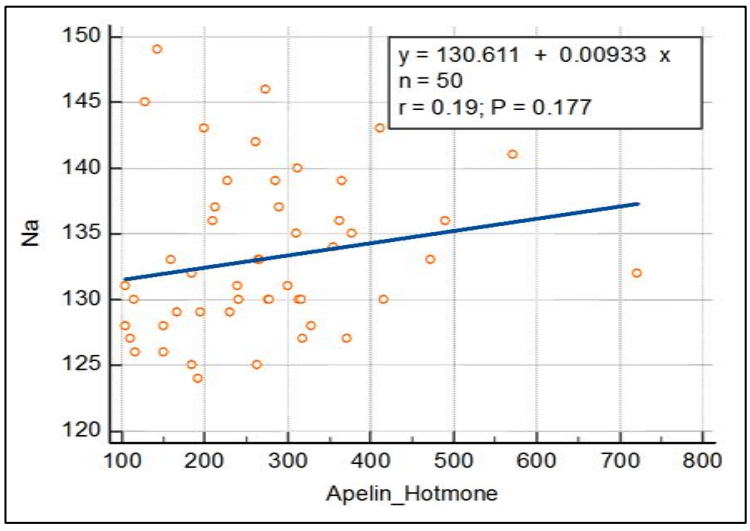

Figure 4.6 Correlation between apelin with Na^+ in patients with CKD

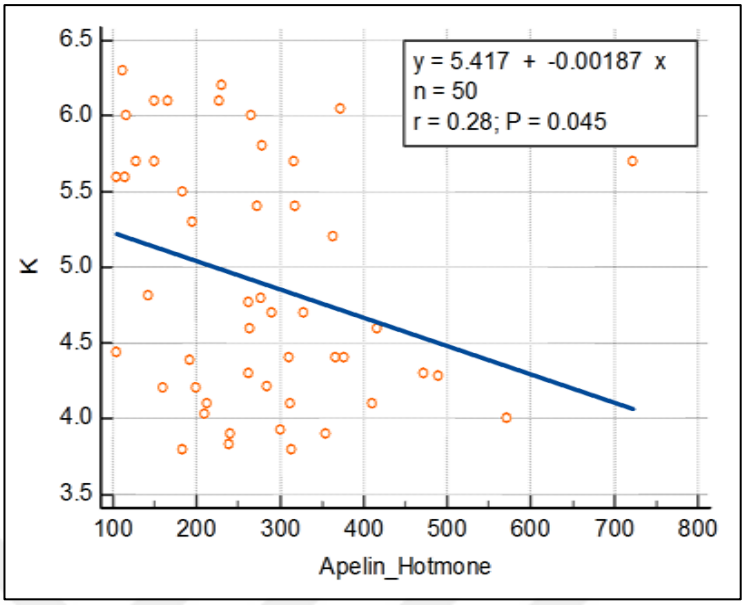

Figure 4.7 Correlation between apelin with K^+ in patients with CKD

4.8 The Result of Albumin Levels

As shown in Table 4.10, mean serum albumin in CKD patients was lower levels than those of the control (3.01± 0.76 g/dL vs 4.46± 0.55 g/dL: P-value=.042) (Figure 4.8).

Table 4.10 The results of albumin in CKD patients and control

Parameters / **Groups**	**Mean±SD**	
	CKD (n=50)	Control (n=40)
Albumin (g/dL)	3.01±0.76	4.46±0.55
Difference	1.4433	
SD	0.6779	
SE	0.1438	
95% CI	1.1575 to 1.7290	
t- Test	10.036	
DF	88	
P value	0.042	
CI: Confidence interval for the difference between the means, t- Test: Test statistic t, DF: Freedom degrees.		

Our findings corroborate those of (Sarhat *et al.* 2018) who found that chronic renal patients had lower levels of total protein, globulin, and albumin activity compared to healthy people. Furthermore, their results are consistent with those of (Doğan *et al.* 2018b).

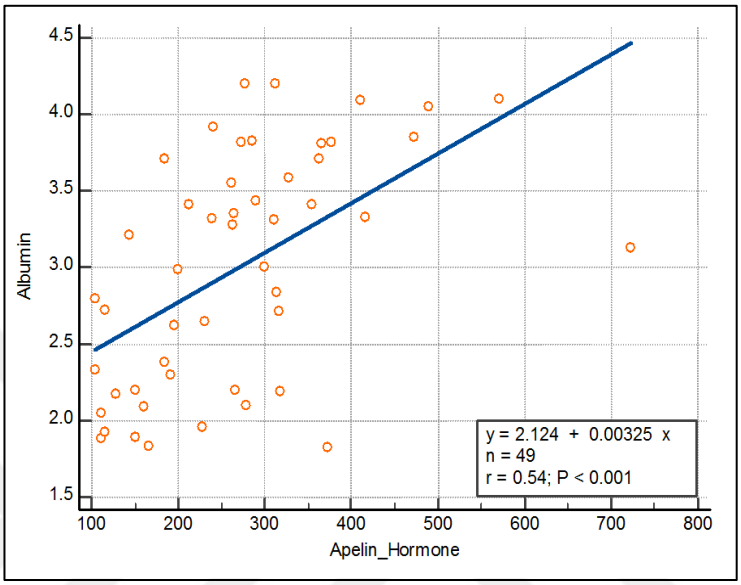

Figure 4.8 Correlation between apelin and Albumin in patients with CKD

4.9 The Result Serum Glucose and Hba1c Levels

In CKD, significantly increased levels of glucose and plasma HbA1c were found compared to healthy control. (108.40±55.73 vs 84.86±9.8 mg/mL; P-value= 0.0097 and 5.43±2.15 vs 4.15±0.36 mg/mL; P-value= 0.0094, respectively), according to Tables 4.11 and 4.12 and Figures 4.9 and 4.10.

Table 4.11 The results of glucose in CKD patients and control

Parameters / Groups	Mean±SD	
	CKD (n=50)	Control (n=40)
Glucose (mg/dL)	108.40±55.7396	84.865±9.82
Difference	-23.6000	
SD	42.1041	
SE	8.9316	
95% CI	-41.3497 to -5.8503	
t- Test	-2.642	
DF	88	
P value	0.0097	
CI: Confidence interval for the difference between the means, t- Test: Test statistic t, DF: Freedom degrees.		

Table 4.12 The results of HbA1c in CKD patients and control

Parameters / Groups	Mean±SD	
	CKD (n=50)	Control (n=40)
HbA1c (%)	5.43±2.15	4.15±0.364
Difference	-0.9157	
SD	1.6255	
SE	0.3448	
95% CI	-1.6009 to -0.2304	
t- Test	-2.655	
DF	88	
P value	0.0094	
CI: Confidence interval for the difference between the means, t- Test: Test statistic t, DF: Freedom degrees.		

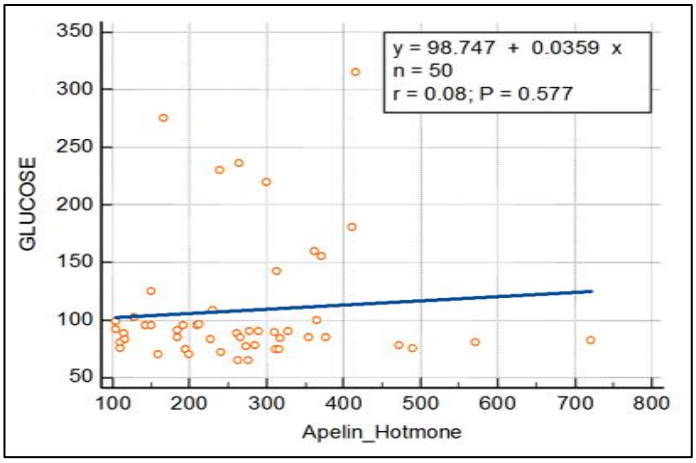

Figure 4.9 Correlation between apelin with glucose in patients with CKD

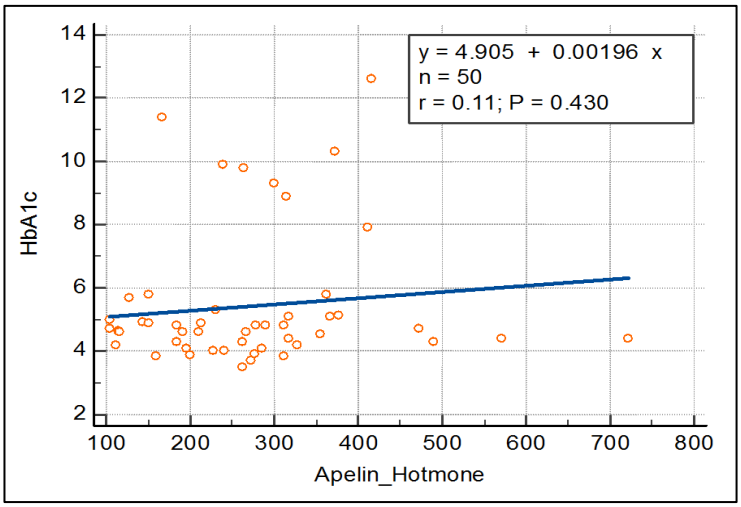

Figure 4.10 Correlation between apelin with HbA1c in patients with CKD

4.10 The Correlation Analysis of Apelin with Parameters Studied in CKD Group

In a correlation study (Table 4.13), apelin was shown to be inversely linked with age, BMI, BUN, creatinine, K^+, and albumin. Apelin and eGFR have a positive correlation (P= 0.001). These results agree with those of (Doğan *et al.* 2018b). The data showing results of all parameters

Table 4.13 The correlation analysis of apelin with parameters studed in CKD patients

Parameters	**Apelin Hormone (n=50)**	
Age	r	0.50
	P-value	0.001
BMI	r	0.36
	P-value	0.01
BUN	r	0.53
	P-value	0.001
Creatinine	r	0.49
	P-value	0.001
Glucose	r	0.08
	P-value	0.577
HBA1c	r	0.11
	P-value	0.43
Na	r	0.19
	P value	0.177
K	r	0.28
	P-value	0.04
eGFR	r	0.45
	P-value	0.001

A range of biological functions are performed by the apelin in KD. Several renal disorders, including CKD, feature a dual role of the apelin system. Apelin was a novel component of the RAS and plays a significant effect on vasodilation, decreasing blood pressure, boosting heart contractility, and other processes. Consequently, there are nuances in the function of the apelin in renal disease (Huang *et al.* 2018).

According to our research results, apelin could serve as a biomarker to determining the severity and development of renal disease.

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

Today, CKD was regarded as one of the main factorscontributing to mortality worldwide, including Iraq. The research study looked at how levels of aplin in the blood are related in people with CKD. The study consisted of 90 patients with CKD at AL-Fallujah Teaching Hospital, AL-Anbar Province, Iraq,

1. Apelin was lowest in CKD patients than control and positively correlated with eGFR.

2. The creatinine and BUN concentrations were significantly elevated in CKD patients than those of healthy control. The levels of BUN and creatinine were inversely associated with apelin.

3. CKD patients had lower mean albumin in than control and correlation analysis apelin was negatively correlated with albumin.

4. CKD patients had lower mean Na^+ levels and higher K^+ levels than controls with negative correlation analysis of apelin and K only.

5. Serum glucose and plasma HbA1c values were significantly elevated in CKD than control, but no correlation analysis was found between them with apelin.

Our findings results suggest that apelin might be a biomarker to assessing the severity and progression of KD.

5.2 Recommendations

Cardiovascular disease was independently linked to CKD. An emerging prospective therapeutic target for numerous disorders was the apelin. Apelin controls glomerular hemodynamics and enhances aquaresis in tubules in preclinical experiments. Apelin was also protective in many kidney damage models. Despite this, Iraq has not yet to consider apelin as a potential treatment option for people with CKD. Since some research suggests that apelin was a potential treatment approach for CKD, we will continue to work on this subject in the future.

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