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EXPLORATION THE RELATIONSHIP OF NETRIN-1 WITH OSTEOPONTIN IN CHRONIC KIDNEY DISEASE PATIENTS

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EXPLORATION THE RELATIONSHIP OF NETRIN-1 WITH OSTEOPONTIN IN CHRONIC KIDNEY DISEASE PATIENTS

By Manhal Ali Mohammed ALBOSODAA

August 2021

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

EXPLORATION THE RELATIONSHIP OF NETRIN-1 WITH OSTEOPONTIN IN CHRONIC KIDNEY DISEASE PATIENTS

Manhal Ali Mohammed ALBOSODAA

Master of Science in Chemistry

Advisor: Prof. Dr. Ayşe ŞAHIN YAĞLIOĞLU

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August 2021

This research objective was to evaluate Netrin-1 and Osteopontin and some other chemical tests in chronic kidney disease patients. This disease is considered a serious disease that poses great risks to the developed population of the country. The main goal was to explore the potential association that might exist between Netrin-1 with Osteopontin and some other chemotherapy test in the patients and the levels of blood urea and serum creatinine. The results of the study indicated that there was a strong correlation between age and K, Na, and Ca, as well as urea and creatinine, as they were considered diagnostic tests for the disease, while there was no association with the gout test, and within the main tests in our research there was a strong correlation between Netrin-1 and Osteopontin with urea and creatinine in chronic kidney disease, which indicates the possibility of using these tests in early diagnosis or even in the follow-up of patients who are believed to have kidney failure or follow-up of those already diagnosed with chronic kidney disease.

2021, 71 pages

Keywords: Netrin-1, Osteopontin, Chronic kidney disease

ÖZET

KRONİK BÖBREK HASTALIĞINDA NETRIN-1'IN OSTEOPONTIN İLE İLİŞKİSİNİN İNCELENMESİ

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Ağustos 2021

Bu çalışma, kronik böbrek hastalığı olan hastalarda Netrin-1 ve Osteopontin ve diğer bazı kimyasal testleri değerlendirmek için yapılmıştırı. Kronik böbrek hastalığı, ülkenin gelişmiş nüfusu için büyük riskler oluşturan ciddi bir hastalık olarak kabul edilmektedir. Bu çalışmanın temel amacı, KBH'li hastalarda Netrin-1 ile Osteopontin ve diğer bazı kemoterapi testleri arasında var olabilecek olası ilişkiyi ve kan üre ve serum kreatinin düzeylerini araştırıyormuştur. Çalışmanın sonuçları, hastalık için tanısal testler olarak kabul edildiğinden yaş ile K, Na ve Ca ile üre ve kreatinin arasında güçlü bir korelasyon olduğunu, ancak gut testi ile bir ilişki olmadığını gösterdi ve Araştırmamızdaki ana testler içinde, Netrin-1 ve Osteopontin ile kronik böbrek hastalığında üre ve kreatinin arasında güçlü bir korelasyon vardı, bu da bu testlerin erken tanıda ve hatta hastaların takibinde kullanılma olasılığını gösteriyor. Böbrek yetmezliği veya kronik böbrek hastalığı teşhisi konmuş kişilerin takibinde olduğuna inanılmaktadır.

2021, 71 sayfa

Anahtar Kelimeler: Netrin-1, Osteopontin, Kronik böbrek hastalığı

PREFACE AND ACKNOWLEDGEMENTS

Thanks to Almighty God, for assisting me in all aspects of my studies and providing me with the strength to accomplish my job. I'd want to include some exciting moments from the study's conclusion on this page, and I'm proud of them. I'd also like to use this time to thank individuals who made this work possible.

Prof. Dr. Ayşe ŞAHIN YAĞLIOĞLU, my thesis supervisor, deserves special thanks for her advice and support throughout the project. Her helpful remarks on the thesis chapters enhance the entire work, and her words of encouragement inspire me to work hard every day. This thesis is the fruit of my collaboration with my supervisor, Prof. Dr. Ayşe ŞAHIN YAĞLIOĞLU, and I want to express my gratitude and admiration to her once again. I'd want to convey my heartfelt thanks to the department's head and all of the department's instructors. Thank you also to Asst. Prof. Dr. Khalid F. AL-RAWI (Co-Supervisor) for his assistance in the lab.

Manhal Ali Mohammed ALBOSODAA
Çankırı-2021

CONTENTS

LIST OF ABBREVIATION

µg	Microgram
ACR	Albumin to Creatinine Ratio
AKI	Acute Kidney Illnesses
BSP-1	Bone Sialoprotein I
CDC	Centers for Disease Control
CKD	Chronic Kidney Disease
CT	Computerized Tomography
DNA	Deoxyribonucleic Acid
ESRD	End-Stage Renal Disease
ETA-1	Early T-lymphocyte Activation
HRP	Horseradish Peroxidase
I-	Iodide
kg	Kilogram
LLD	Lower Limit of Detection
m^2	Square meters
mL	Milliliters
mm	Millimeter
ng	Nano gram
NHS	National Health Service
^oC	Degrees celsius
OH	Phenolic Hydroxyl
PKD	Polycystic Kidney Disease
SKI	Severe Kidney Illnesses
SPP1	Secreted Phosphoprotein 1
WHO	World Health Organization

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1. INTRODUCTION

Chronic kidney disease (CKD) is a global issue that the health centers have been encountered. Different serious problems can result from the disease, for instance the kidney failure, sometimes cardiovascular disease, and probably death of the premature (Levey *et al.* 2005, Coca *et al.* 2012, Di Iorio *et al.* 2012). Therefore, it is essential to define and classify kidney disease for international implementation of a practical-treatment guidelines. CKD, however, can be considered as a serious condition when happen some tardiness of the function of renal gradually takes place in some years. In some cases, kidney transplant becomes needed. In case of left untreated, it can progress to the End-Stage Renal Disease (ESRD) and dialysis treatment. Patients suffers of CKD can experience bad life quality, rising of health care expenses, and increment of death.

CKD can also be illustrated as either by decrease of glomerular filtration amount into kidney spoilage, for instance, proteinuria, hematuria, or non-normal biopsy. Therefore, these eccentricity activities need to be presented maximum of least three months so that can be considered as CKD, if not it would be thought as an acute kidney damage (Schoolwerth *et al.* 2006, Brosnahanand and Fraer 2010).

Netrin-1, however, is a one of the axonal that is a member the family of guidance protein; it can be both negative and positive regulator inside the nervous system. This protein is a one of the leukocyte-guidance molecules. By activating its receptor, Nertin-1 can prevent the monocyte migration, neutrophils, and lymphocytes (Mirakaj *et al.* 2010, Ly *et al.* 2005, Rosenberger *et al.* 2009). By acting through Unc5b receptor, Netrin-1 may constringe a renal ischemia issue and its related inflammation. Besides, Netrin-1 down regulation of the cells of vascular endothelial can active endothelial cells and infiltrate into leukocytes (Wang *et al.* 2008, Tadagavadi *et al.* 2010).

Netrin-1, furthermore, occurs on vascular endothelium, usually dominant by the infect cytokines. It can prevent basal cell movement towards tissue surfaces. Moreover, it's down regulation at the inflammation outset will booster leukocyte construction. Some

findings have concluded that Netrin-1 helps the inflammation of chronic; macrophages in the atherosclerotic tissue of plaques, and obese in the tissue of adipose, which then results in macrophage survival, and more chronic inflammation (Ly *et al.* 2005, Van Gils *et al.* 2012, Ramkhelawon *et al.* 2013, Ramkhelawon *et al.* 2014). Within the living of Netrin-1, controlling the inflammation leads to the need of examination of its potential function in bone metabolism in the patients that suffered from CKD.

The main objevtive of this study is to explore the possible association that might present between Netrin-1 with Osteopontin in the CKD patients. This study will be one of the few ones in this field. Thus, it will be a valuable contribution to the literature.

2. LITERATURE REVIEW

This chapter consists of three major items, namely: general information, the execration system, and the diagnosis and biochemical tests.

2.1 The Execration System

Excretion is the removal action operated by the kidney of toxic wastes from the body. These wastes are usually produced from two major sources, namely: Carbon dioxide and Urea. Carbon dioxide is implemented during respiration. However, the urea is mainly outputted by the decomposition of extra-water proteins arrives to the body liver. It is of paramount important to dispose of these wastes from the human's body, since their accumulation can be a serious poison and may harm the body. There are different organs which removes waste from the body. Two main organs that work on the waste removal from the body, are existed in the human bodies, which are lungs and kidneys (Lamb *et al.* 2005, Myers *et al.* 2005). In this study, a focus is made on the kidney process and its functionality.

2.1.1 The kidney

The excretory system of human bodies receives the liquid wastes of the body and works of their removal. These main organs are: Two kidneys, two ureters, Bladder and Urethra. The kidneys, however, look like bean-shaped organs towards the back of our body where above the body waist. People are born with two kidneys. Blood consistently comes to the kidneys around the time. The kidney artery puts in the dirty blood that having waste substances towards them. Therefore, the function of kidney is to dispose of the toxin substance urea as well as other waste salts and excess water from the blood and change their structure into of yellow-liquid which is named by urine. The blood that is cleaned is then transformed from the kidneys by the renal vein.

2.1.2 The anatomy of kidney

The kidneys are similar bean-shaped work on filtering the minerals, maintaining fluid balance, disposing of wastes, and controlling blood volume. They are situated on both sides of the spine, in the retroperitoneal space. The left one is usually located a little-bit higher comparing to the right one. This due to the fact that the liver is located on the right side of the abdominal cavity above the kidney. Each kidney has around 125 to 175 grams weight for males while the females range is 115- 155 (in grams). The kidney length is about 11 - 14 cen. while 6 cm for width and 4 cm of thick (Levey *et al.* 2003).

It is bounded by a tough fibrous renal capsule are each kidney. Beneath of that fibrous, there are two layers of fat serve. The adrenal glands are located on the top part. Each of them has an outer renal cortex and an inner renal medulla, where the Nephron passing through them. The Blood arrives the kidneys in the renal arteries and goes out of the kidney from the renal veins. Although the kidneys are comparatively small organs, their blood portion is around 25% of the heart (Sands and Verlander 2005, Clark *et al.* 2009, Collins *et al.* 2009). An illustration example of the human kidneys is shown in Figure 2.1.

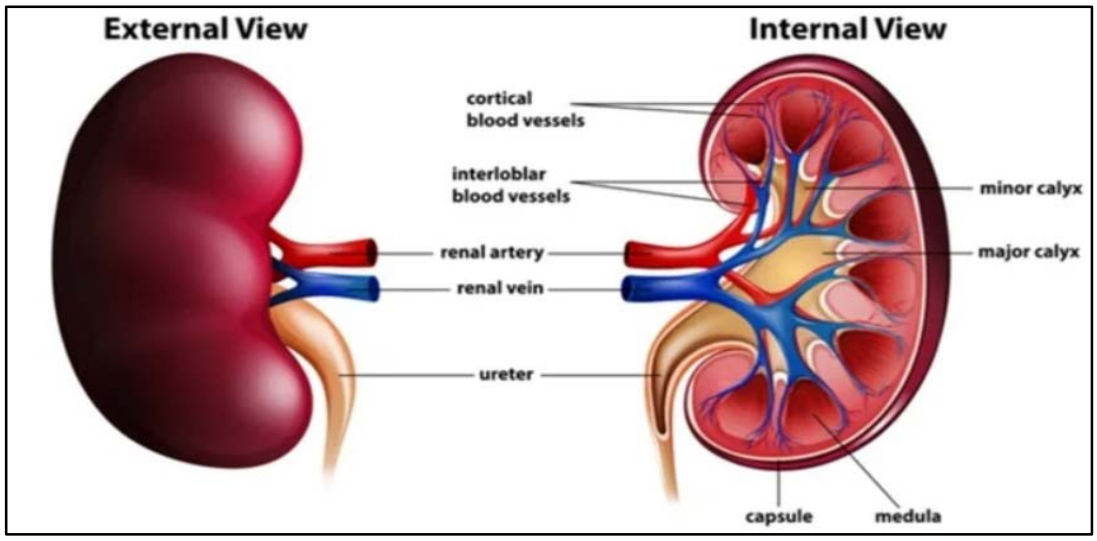

Figure 2.1 Diagram of the human kidney anatomy (Sands and Verlander 2005)

2.1.3 The type disease of the kidney

In general, there are different kinds of existing kidney diseases exist. Acute kidney injury usually comes from a build-up of waste minerals in blood. This leads to hardness in controlling the right balance of fluid.in addition, it can affect other organs in the body, like the brain, heart, and lungs. These are the results of a decreased blood flow, direct damage to the kidneys and blockage. On the other hand, Kidney Failure takes place when the kidney gets the inability to filter the waste from the blood. Kidney failure usually causes from loss of blood flow to the kidneys, urine elimination issues, infection, hemolytic uremic syndrome, drugs and alcohol, certain antibiotics, chemotherapy drugs, *etc*. It can result in diabetes, high cholesterol, kidney infections, glomerulonephritis, and others (Salahudeen 2003, Lopes *et al.* 2003, Shinaberger *et al.* 2006, Chan *et al.* 2007, Mehrotra 2011). In the next sections, more details will be presented about CKD as it is the main part of this study.

2.1.4 Chronic kidney disease

2.1.4.1 Overview

It is identified as a main cause of early cardiovascular disease according to the World Health Organization report in 2016, and may lead to the cause of death worldwide. Additionally, the WHO 2016 Report indicated that CKD is the 12th leading cause of mortality. The seventeenth cause of global disability. Similarly, a critical connection between CKD and increased hospitalization, morbidity, and mortality has been found lately (Humphreys *et al.* 2012).

Undeniably; furthermore, CKD is a severe disorder, particularly when the tardiness of the function that happen in renal progresses gradually over a period of several years. Additionally, it can be diagnosed by indications such as cardio-vascular disease, hypertension, diabetes, and obesity. Without therapy, it can progress to End-Stage Renal Disease, necessitating dialysis or a kidney transplant.

On the other hand, CKD can also be illustrated as either a minimizing in the glomerular filtration level or by the signal of or kidney damage disfunction. CKD are the results of different factors comes from many heterogeneous disease pathways that change the kidney functionality. It is noticed by Eggers in 2011 that around two million people globally require renal replacement to stay alive normally although it is commonly it is 10% less for those requiring it. Eggers also glimpsed that the number would keep rising due to the fact that more diabetes prevalence. CKD is the major factor of premature mortal and big economic amount for sectors in addition to the private and public (De Lusignan *et al.* 2005).

The global and regional death rate out of 235 causes of death for 20 years old are categorized between in 1990 and 2010 (Lozano *et al.* 2012). The study has also shown that the mortality in the diabetes and are around twice globally in 2010 comparing to the mortals from period before.

In North America, it was found that around 19.2 million Americans have CKD (Schoolwerth *et al.* 2006). For adults, the crude prevalence found of 15.1% of US population. The propagation stage of the disease was estimated as: 5.7% of stage 1. For stage 2 and 3, it is about 5.4%. 0.4% for stages 4 and 5.

In the same manner, CKD is serious mortal problem in the United Kingdom. On average, over 5% of the UK people have CKD. In 2009 around 49,000 people got renal replacement therapy, which is estimated as an increment number by 3.2% for those in the previous year (Steenkamp *et al.* 2011).

2.1.4.2 Symptoms

CDK symptoms are different from stage to stage. Therefore, it is of paramount important to define the CKD stages before referring to the symptoms. Based on measurement or estimation, Levey *et al.* 2003 has categorized CKD in five stages as shown in Table 2.1.

Table 2.1 Classification of the CKD Stages (Vijayarani and Dhayanand 2015, Webster *et al.* 2017)

Stage	Glomerular Filtration Rate	Description	Treatment stage
1	90+	Normal kidney function but urine findings or structural abnormalities or genetic trait point to kidney disease	Observation, control of blood pressure
2	60-89	Mildly reduced kidney function, and other findings (as for stage 1) point to kidney disease	Observation, control of blood pressure and risk factors
3A	45-59	Moderately reduced kidney function	Observation, control of blood pressure and risk factors.
3B	30-44	Moderately reduced kidney function	Observation, control of blood pressure and risk factors.
4	15-29	Severely reduced kidney function	Planning for endstage renal failure.
5	<15 or on dialysis	Very severe, or end stage kidney failure (sometimes called established renal failure)	Treatment choices.

People are with not symptomatic or non-specific, for example lethargy, itching, or no appetite. Diagnosis is usually taken place the results of checks. Sometimes, the diagnosis is made when symptoms become severe. The common indicator of the kidney functionality measured by exogenous markers, or evaluated by some models' equations (Webster *et al.* 2017). Samples of Kidney biopsy can show a good indicator CKD, is a common change like sclerosis of glomerular or interstitial fibrosis. Some other symptoms can be found in the advance complications of CKD caused by decreased erythropoietin production of by the kidney; decreased red blood cells and iron low level; and mineral bone due to less vitamin D. Interference or medications for the symptoms, helps the CKD patients to be alive with the disease.

2.1.4.3 Causes

The causes of CKD are different and has a big variation and diverse. Some of the CKD causes are more usual than others. Glomerulonephritis, for example, is the cause of 16%

of US patients with UK (Chen *et al.* 2010) and 18% of cases in patients < 65 years in the UK. However, the other common causes are immune diseases (systemic lupus erythematosus and diabetes mellitus), infectious diseases (such as hepatitis B), drugs and poisons, non-steroidal anti-inflammatory agents and penicillamine, tumors and renal transplantation (Kalantar-Zadeh *et al.* 2006, Farrington *et al.* 2009, Steiner *et al.* 2009).

Another cause of chronic kidney disease in individuals is diabetic nephropathy. Proteinuria of more than 0.5g is considered diabetic nephropathy. End-stage renal disease is caused by a sustained decline in kidney function caused by albuminuria. These individuals are also at an increased risk of cardiovascular disease when their renal function deteriorates (Laudanski *et al.* 2013, Koo *et al.* 2005, Evans and Forsyth 2004).

Secondary hypertension is caused by an underlying condition. Secondary hypertension, which is caused by CKD, endocrine, cardiovascular, pregnancy, and medication usage, accounts for fewer than 10% of all instances of hypertension (Tanyel 2000, Kerr *et al.* 2012, Chobanian *et al.* 2003).

2.1.4.4 Risk factors

CKD is a chronic disease that is hard to be remedied. It is caused by complex diabetes and hypertension, and it can lead to death. Kidney disease affects 5 to 7% of the population in various nations as a result of poor health caused by diabetes and hypertension (Couser *et al.* 2011, Chen *et al.* 2010, Bergmann *et al.* 2018).

Factors make more happen for chronic disease existence, like CKD. They comprise unhealthy styles mode, deteriorating economic status, and less access health care. It is a relation between ages, nephrotoxic agents' usage, diabetes, for CKD (Wilkinson 2012).

Infectious diseases were also found classified by a risk factor next to bad sanitation, bad clean or saving the supply of water, and high levels. Pesticide exposure, dehydration, and

alcohol use were shown to be associated to predominance among males under 60 years old, with less heavy metals. Risk factors are hypertension for progressive glomerulosclerosis. It also includes smoking. The starting activation of Glomerular microinflammation leads to endothelial cells of hypertension, with inflammatory cells activating mesangial cells to proliferate. The mesangioblasts are able to produce a matrix of extracellular, results in mesangial expansion of glomeruloscelrosis (Morton *et al.* 2016, Okaka and Ojogwu 2012, Reddy 2015, Dwivedi *et al.* 2011).

2.1.4.5 Complications

Figure 2.2 shows a diagram of the CKD complications. The shaded ovals show the stages, whereas the unshaded ovals are their complications. The more arrows indicate the risk factors of initiation and progression. Intervene for a stage is given below each stage. CKD risk factors are generally examined in those who have no symptoms. People who are at a higher risk of developing CKD should be evaluated. The word "complications" refers to all CKD and treatment-related issues, including hypertension, anemia, malnutrition, bone disease, neuropathy, and reduced quality of life. As kidney disease advances, thicker means more problems, which means a higher chance of consequences (Kautz *et al.* 2003, Levey and Coresh 2003, Evans and Johnson *et al.* 2004).

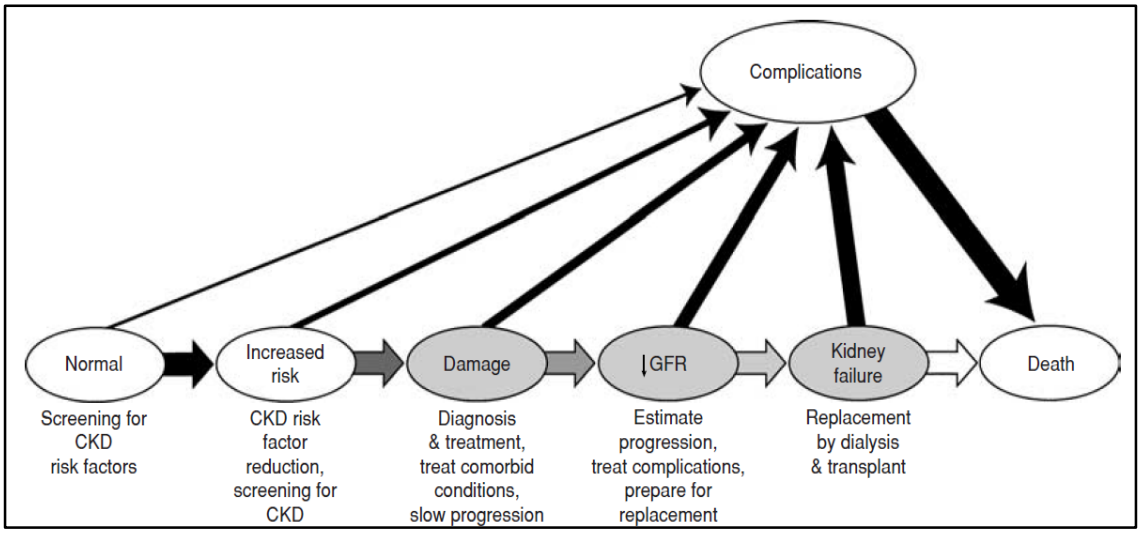

Figure 2.2 Diagram of the CKD complications (Levey *et al.* 2005)

2.1.4.6 Prevention

Diseases could be prevented when there is knowledge its risk factors. Less awareness leads to difficulties for people get health care to test the functions. Test of the functionality by the awareness and screening may allow finding factors for the type life. Also, it includes the dietary altering early to avoid the kidneys from getting worse (Jain 2014).

Centers for disease control in 2016 found that prevention factors are the most effective way to less patients suffering and financial bills. Reducing of prevention CKD prevalence, with CKD risk factors the effectiveness of preventive programs.

Early detection through screening the risky patients is also a cost effective in CKD prevention. Strict blood pressure management, proteinuria reduction, hyperlipidemia control of recognized high-risk populations, and early intervention of CKD complications by primary care doctors might all be slowed by the progression. As a result, primary care doctors request CKD testing to ensure that they are providing quality care to their patients, as they are well-versed in the disease (Pagels *et al.* 2012).

2.1.4.7 Diagnosis and treatment

It is critical to evaluate the sort of functional difficulty that the patient has in order to provide quality health care at various stages of their condition. The ill patients have various aetiologies, and while their drugs are generally comparable, the therapies available, such as continuous ambulatory peritoneal dialysis transplantation and conservative therapy, are extremely varied in terms of delivery and efficiency (Coresh *et al.* 2007).

Sudden end of life (death) can strike at any time, without warning or diagnosis. The cost of therapy is a concern that may be addressed by using social media to solicit donations from the general population. Furthermore, enabling is the result of undergoing dialysis or

being able to finance kidney transplant. Due to financial constraints, 1,000 individuals with CKD are on dialysis (So *et al.* 2018).

The treatment, moreover, for primary prevention with statins events results in mortality. The treatment of low-risk were an issue. In general, they reduce the revascularization need. Diabetic patients with nephropathy having more issues. There are many facts show that the early detection and prevention of CKD. In which, the early interventions can slow progression of CKD patient conditions, as well as a good treatment of illnesses persons with diabetes (Kamoun *et al.* 1997). However, studies have suggested the use of Netrin-1 and Osteopontin proteins as remedies for the CKD patients. Therefore, we devoted this research to study the relationship of them with the CKD patients and its effectiveness.

2.1.4.8 Nephrotic syndrome

The nephrotic syndrome is another disease that can affect both kids and adult kidney disease. It is a very common type of the disease that recently shown in kids. Roelans identified the clinical description of nephrotic syndrome. Later Zuinger went into further depth regarding the disease's clinical history and its significance as a cause of chronic failure. Nephrotic syndrome, on the other hand, is characterized by a higher level of proteinuria. The more clinical features are also usually indications, such as hypoalbuminemia, and hyperlipidemia (Cameron 2002, Jayawardene *et al.* 2002, Doe *et al.* 2006).

The disease is a result of more structural functional issue in the barrier of glomerular filtration. It causes a decrease in the capacity to eliminate urine protein. In terms of physiology, the liver compensates for the enormous loss by producing more protein and lipoprotein. Nephrotic syndrome occurs when protein loss in the urine causes an increase in albumin production in the liver, resulting in hypoalbuminemia and edema. Nephrotic syndrome can be caused by a number of glomerular and systemic illnesses, however idiopathic nephrotic syndrome affects the majority of children. Nephrotic syndrome

increases mortality by 67 percent, generally as a result of infections (Doe *et al.* 2006, Sadowski *et al.* 2015, Tsai *et al.* 2016).

The presence of edema and severe proteinuria of more than 40 mg/ m^2/hr is required for the diagnosis of nephrotic syndrome. Hypoalbuminemia (2.5g/dL) and a urine protein/creatinine ratio > 2.0mg/mg are other signs of the illness. Furthermore, for three days in a row, the Remission indicates a significant decrease in proteinuria of about 4 mg/ m^2/ hr or a urine albumin dipstick of 0 to trace. Furthermore, it occurs with the clearance of edema and a serum albumin level of at least 3.5g/dL. Finally, nephrotic syndrome might manifest itself in a variety of ways across various healthcare settings. Patients with nephrotic syndrome should seek the advice of a local renal expert before proceeding with additional testing and therapy (Salomon *et al.* 2013, Crew and Appel 2004).

2.1.4.9 Acute kidney disease

It is another syndrome developed by a quick badness of kidney function. AKI is usually testes by the founding of illness in patients. The consequences are the amount of summing of waste of products, electrolytes, and fluids. It can also lead to fewer effects, such as less immune and non-renal organ dysfunction. The effect depends on the influences and the site. It is usually linked with short and long complications, such as an increased mortality and morbidity. Nowadays, AKI is considered as a public issue; where it's test and detect are essential to examine the sickness kind (Lewington *et al.* 2013, Mehta *et al.* 2015).

The diagnosis built on amount in serum keratinize and the fall in the urines; if the serum keratinize above 0.3 mg/dL or increase 48 hr; or above 1.5-fold in seven days. A summary of the definition and classification of AKI is given in Table 2.2. However, AKI stages are characterized by the alteration of serum keratinize or urine, as follows (Fliser *et al.* 2012, Ostermann *et al.* 2012, Kellum *et al.* 2015):

1. Rise in keratinize of the serum to ≥ 0.3mg/dL in 48 hr.
2. Rise in keratinize of the serum of ≥ 1.5 times.
3. Urine volume.

Table 2.2 Classification of AKI stages (Barry and James 2015, Chaturvedi *et al.* 2017)

AKI stage	Serum creatinine criteria	Urine output criteria
AKI stage I	Increase of serum creatinine by ≥0.3 mg/dl (≥26.4 μmol/L)	<0.5 ml/kg/h for 6–12 hr
AKI stage II	Increase of serum creatinine to 2.0–2.9 times from baseline	<0.5 ml/kg/h for ≥12 hr
AKI stage III	Increase of serum creatinine ≥3.0 times from baseline	<0.3 ml/kg/h for ≥24 hr

2.1.4.10 Polycystic kidney disease

It is a failure of the kidney by the growth of cysts of numerous. PKD cysts may increase the kidneys when changing the structure, leading to less kidney function and maybe to failure, as shown in Figure 2.3. PKD takes places in some years. The patients need test of transplantation. About 50% of people have PKD and can develop failure. PKD may result in issues in the liver. The cysts number can distinguish usually as harmless cysts in the late stage of kidney life (Audrézet *et al.* 2012).

Pain in the back and sides, as well as headaches, are common complaints. The discomfort can be short-term or long-term, and it can range from moderate to severe. The following problems can occur in people with PKD (Nevis *et al.* 2011):

• Infections of the urinary tract in kidney cysts
• Heart valve problems
• High blood pressure
• Kidney stones

Diverticulitis is a condition in which tiny pouches protrude outward from the colon.

The imaging method is used to make the diagnosis. Apart from magnetic resonance imaging, computerized tomography (CT) scans are the most frequent type of diagnostic picture. Damage and development can take on many forms depending on the circumstance. The outcome may vary depending on the patient's age. Finally, a genetic test that detects mutations is used to determine the diagnosis. This is generally done for autosomal genes (Pope 2012, Collins 2013).

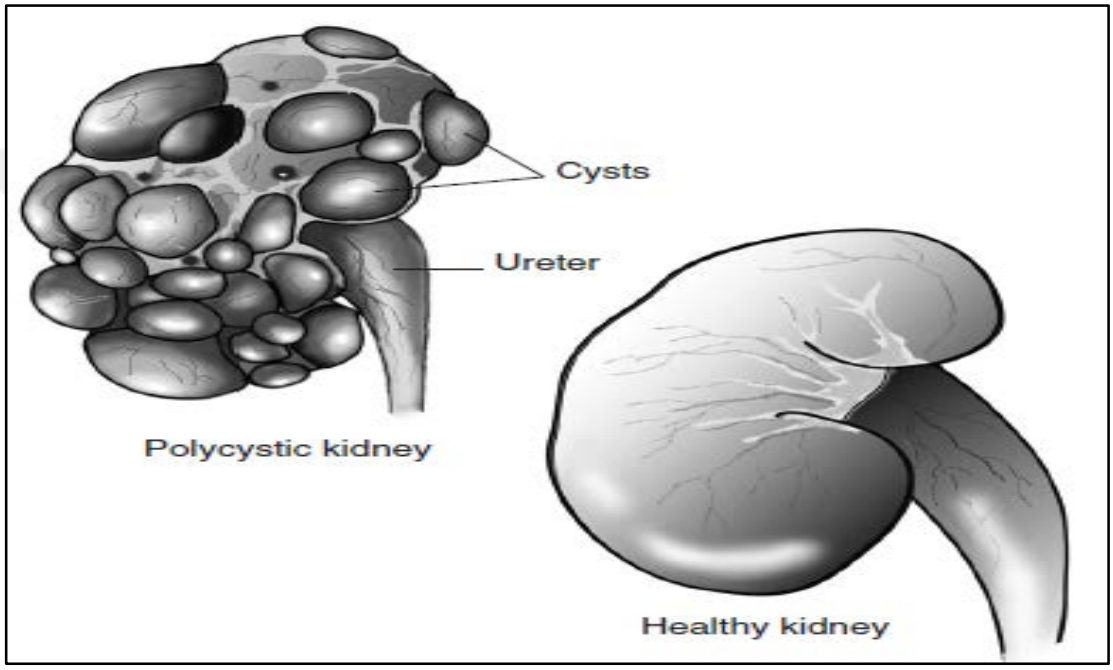

Figure 2.3 Health and polycystic kidney (Bergmann *et al.* 2018)

2.2 The Diagnosis and Biochemical Tests

The establishment of the finding of kidney disease is difficult because of different aetiologias, and the ability at the early occurrence. The cause is due to the pre-renal, intrinsic renal disease, post-renal, and hyperplasia. However, many different biochemical markers are existing which are marked of the function. Others are determined to assess the impact of the function and developments.

Other markers are applied to find the filtration rate of glomerular. It defines the plasma volume of disposed of a particular analyze over the time. It is produced at a constant rate by the body, freely filtered at the glomerulus without being secreted or reabsorbed by the tubules, and does not undergo extrarenal elimination when the urea is found to be a poor marker, it is measured at variable rates and marks as a reabsorption by the tubules, and the level is influenced by other conditions when the urea is found to be a poor marker, it is measured at variable rates and marks as (Koenig *et al.* 2005, Seegmiller *et al.* 2018, Kooman 2008).

The kidneys have a variety of tasks in life, including filtering metabolic wastes and toxins from the blood, performing endocrine functions, and regulating the composition of the extracellular fluid. It can be difficult and costly to evaluate these functions. As a result, a function marker is used. To locate the phases, keratinize is used. If the abnormalities have persisted for more time, most probably more than three months, the urine albumin content is used. Although have a number of disadvantages, Exogenous substances results are the accurate such as the availablity (Stevens *et al.* 2009, Burballa *et al.* 2018, Lamb *et al.* 2018). Others including Netrin-1 can also be implemented. Details of Netrin-1 are given in detail in the next part.

2.2.1 Netrin-1

It is diagnostic biomarker identifying the CKD. In which, Netrin-1 levels in urine can show the level of kidney damage. As the Netrin-1 is a laminin identified in different organs. It can find its value of renal injury. Different studies showed various Netrins roles of axonal guidance such as: lung and chemoattraction of endothelial cells. The kidney is a high levels in the expression of netrin although is still hard to measure. Therefore in the companion research, we will discuss the structure of the hat netrin-1 is and its role in the human body.

2.2.1.1 Structures of netrin-1

Netrin-1 has a stiff elongated structure with two receptor-binding sites on opposing ends. These two receptors work together to bring receptor molecules together. Two different topologies are visible in the receptor complexes: a 2:2 heterotetramer and a continuous ligand assembly. Different lengths of the linker linking receptor domains fibronectin type III domain 4 (FN4) and FN5 differ between DCC and neotenic splice variants, resulting in a basis for various signaling outputs (Barallobre *et al.* 2005, Han *et al.* 2002, Herzog *et al.* 2007).

Figure 2.4A depicts the structure of the nitrin complex. Two nitrin molecules are in the core of the complex, forming an X-shaped binary that interacts over a broad interface. Dipole is made up of two neotinic molecules with receptor molecules that are parallel to each other and C-terminals that are oriented in the same direction as the neuronal membrane. The two receptor sites are roughly 90 degrees apart at one end of the nitrin structure, but only around 55 degrees apart between the FN4 and FN5 neotinic domains' nitrin binding surfaces. As a result, nitrin must engage with two distinct receptor molecules due to its two receptor binding sites. Normal conformational variations in netrin do not impact receptor interaction; bound and unbound nitrin structures can have a root mean square deviation of 0.9 across 353 Ca atoms. The canonical FNIII foldable structure is shared by Neothenic FN4 and FN5 regions, which are organized linearly with a connection between them in a fully extended form. In the lack of attachment, this junctional area is characterized by flexibility (Doi *et al.* 2006, Mishra *et al.* 2004, Parikh *et al.* 2005).

In addition, there are several peripheral hydrogen bonds and a salt bridge, as shown in Figure 2.4B. The LN Ca^{2+} binding site is near interface number 1, and bound Ca^{2+} would require preserving the proper conformation.

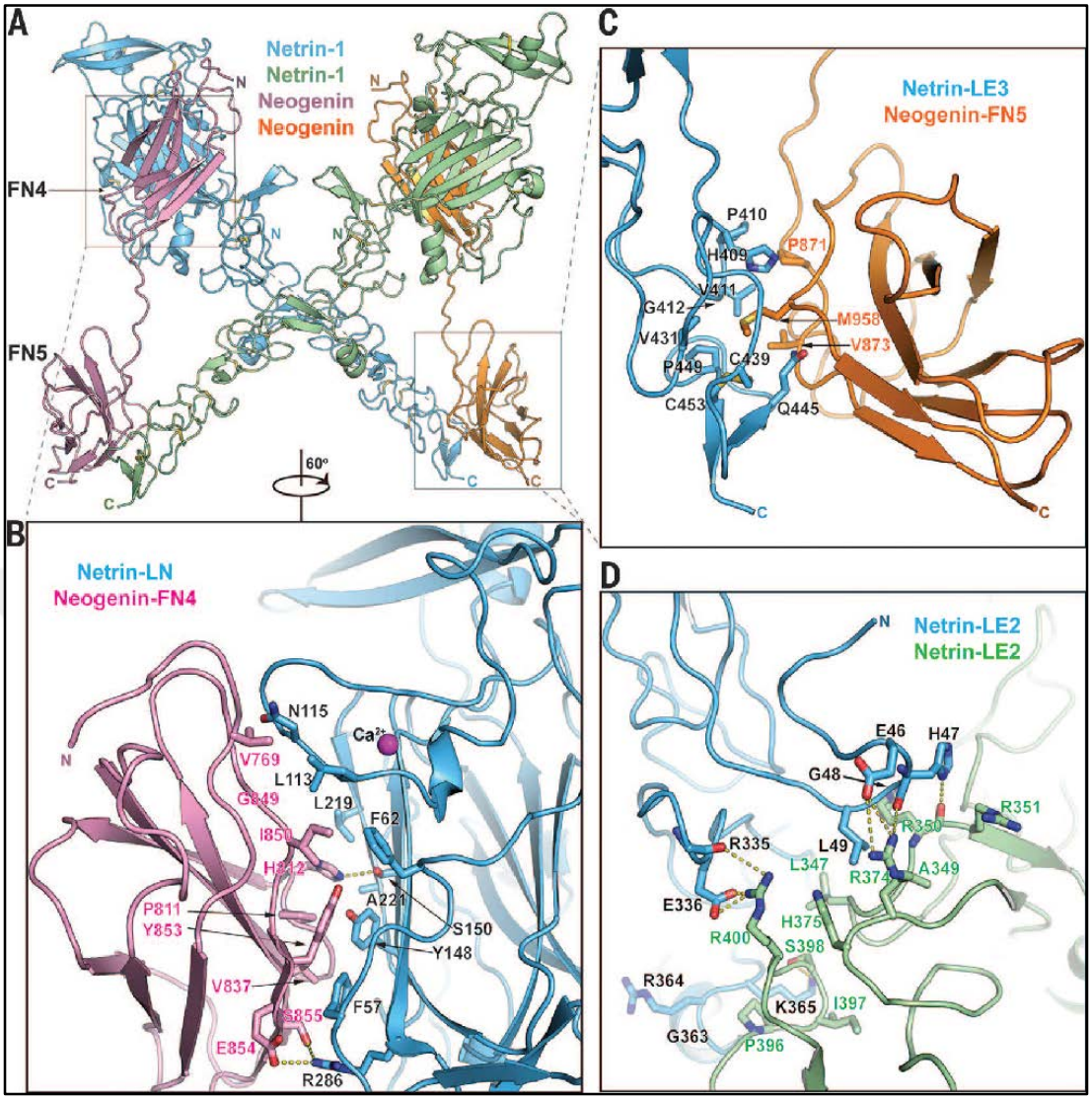

Figure 2.4 Structure of the netrin-1/ neotenic complex (Xu *et al.* 2014)

2.2.1.2 Netrin-1 and role in human body

Among the netrins, Netrin-1 has a lot of research. The gene NTN1 on chromosome 1 encodes it in humans. The conserved 604 amino acid protein is also mentioned. Netrin-1 is a protein that is available in variety kinds for both cells and organs. Netrin-1 expression is high in the brain, heart, kidney, and lungs, but low in the liver, gut, and spleen, according to relative mRNA.

Because of its many activities in tumorgenesis, such as inhibiting macrophage recruitment, promoting cell survival, and stimulating invasiveness, Netrin-1 is classed as an oncogene. Different forms of netrin-1, on the other hand, are elevated in glioblastoma, medulloblastoma, and other cancers, with overexpression associated with a worse prognosis. Cancers produce an excessive quantity of netrin-1, which inhibits their apoptotic pathway (Zhong *et al.* 2019, Bochkov *et al.* 2010).

The functioning is linked to two types of receptors: DCC and its orthologue neotenic, as well as UNC5 receptors (UNC5a-d). They are in charge of triggering the ligand's biological functions. In the presence of netrin-1, cell proliferation, migration, and survival are all boosted. Apoptosis, additionally, occurs in the absence of the ligand (Chistiakov *et al.* 2017, Moore *et al.* 2011).

Through Tits UNC5b receptor, Netrin-1 is crucial in modulating leukocyte movement. Although the exact signaling mechanism is unknown, there is significant evidence that it plays a role in leukocyte recruitment and inflammation. The effects of netrin-1 in the development of atherosclerosis have been found to have both pro- and anti-inflammatory functions in the disease. The temporal and geographical expression of Netrin-1 can explain the contradictory data. Endothelial-derived netrin-1 appears to have a protective impact, but macrophage-secreted netrin-1 appears to have a pro-atherogenic effect within the atherosclerotic plaque (Feinstein *et al.* 2017, Yurchenco *et al.* 2014).

Macrophages, on the other hand, are expressed in Netrin-1. The effects of macrophage-derived netrin-1, on the other hand, may affect macrophage migration in response to chemokines. According to immunohistochemical and quantitative research, only UNC5b is found in monocytes, granulocytes, and lymphocytes (Kappler *et al.* 2000, Moore *et al.* 2011). Because of the aforementioned causes, the present research focuses on the link between Netrin-1 and Osteopontin in renal disorders.

2.2.2 Osteopontin

Osteopontin is a phosphoprotein that has been linked to immune system and tissue remodeling. Acute and chronic diseases can cause an increase in the Osteopontin protein in the heart. This enhanced expression is thought to have a role in fibrillogenesis, which is followed by certain diseases. The increase of Osteopontin is linked to heart failure in this study. The function of Osteopontin in the formation and progression of dilated cardiomyopathy has lately been examined, and several publications have revealed a direct involvement for Osteopontin in advance failure of the heart (Stawowy *et al.* 2002, Renault *et al.* 2010, Satoh *et al.* 2005). Despite this, no study in the field of CKD has been identified that discusses it. As a result, the structure of Osteopontin and its significance in the human body will be discussed in depth in the following sections.

2.2.3 Structure of osteopontin

Osteopontin is a phosphorylated extracellular matrix glycoprotein that has a role in cell signaling by binding to integrins and interacting with development of hormones, cytokines, chemokines, and proteases. It is known as hiden phosphoprotein 1 and it has a function in inflammation and immune system interactions. Osteopontin is a negative acidic hydrophilic protein produced in a number of tissues and cells before being released into all bodily fluids. Alternative splice sites exist, but their functional relevance has yet to be determined. Osteopontin is 314 amino acids in length and has a molecular weight of 32 kilo Daltons (Denhardt *et al.* 2001, Giachelli *et al.* 2003).

The protein Osteopontin is conserved in animals. The nucleus arginine -glycine -aspartic acid -aspartic acid -aspartic acid arginine-glycine arginine-glycine -aspartic acid arginine-glycine -aspartic acid arginine-glycine -aspartic acid. It was discovered to interact with the integrins v1, v3, and v5. Osteopontin interacts with integrins 51 and 91 in a way that is not dependent on the RG-D motif. This connection is mediated by the cryptic integrin binding motif S-V-V-Y-G-L-R, which becomes available to integrins when the enzyme

thrombin cleaves Osteopontin. Cleavages of thrombin reveal an extra integrin binding site, as well as functional chemotactic fragments (Hoyer *et al.* 2001, Davidoff *et al.* 2004).

Osteopontin thrombin cleavage was discovered to occur following blood activation in the pathway of the coagulation. It is thought to occur in regions of tissue injury and cancers, though. Osteopontin can bind to and signal through the CD44 receptor in an RGD-independent manner (Jono *et al.* 2000).

2.2.4 The role of osteopontin in humans body

The protein osteopontin is involved in a variety of biological activities. In vitro bone resorption is promoted to a greater extent by high-phosphorylated recombinant than by unphosphorylated recombinant, for example. Phosphorylated is detected in a combination with the extracellular matrix protein fibronectin in kidney cells. Furthermore, a decrease in glycosylation may result in a decrease in cell surface localization, whereas thrombin proteolytic cleavage improves its adhesion properties and interactions with different integrins (Denhardt *et al.* 2001).

The function of osteopontin is to regulate both normal and pathological mineralization. Osteopontin protein is expressed in normal bone tissue by the cells responsible for bone remodeling, osteoblasts and sometimes osteoclasts. Osteopontin has a function in preventing the development of hydroxyapatite. Osteopontin has been discovered to impact the process of bone resorption rather than bone growth (Renault *et al.* 2010, Singh *et al.* 2010).

Osteopontin, too, plays a crucial role in the inflammatory response. During acute or chronic kidney illness, epithelial and endothelial cells with non-resident macrophages and T cells can produce Osteopontin. Although it is unknown if Osteopontin has pro-inflammatory or anti-inflammatory effects, it is known that it affects macrophage and T cell recruitment. The infusion of neutralizing anti-Osteopontin antibodies can prevent macrophage infiltration caused by osteopontin injection. T lymphocytes respond to

Osteopontin via chemotaxis, activation, and proliferation. Furthermore, thrombin-cleaved Osteopontin fragments are considered to be a function of these features in the original molecule (Matsui *et al.* 2004, Lin *et al.* 2000).

Osteopontin, however, can have a cell-survival effect. It can protect cells from apoptosis by attaching to the v3 integrin on endothelial cells, according to the suggested mechanism. This causes NFB, a pro-survival transcription factor, to become activated. As a result, they prevent apoptosis in the cells. This route is thought to play an essential function in a variety of cells, including kidney tubular epithelial cells (Lenga *et al.* 2008, Weintraub *et al.* 2000).

To conclude, Osteopontin has a wide range of functions. It has the ability to function as both a soluble cytokine and a matricellular protein. As a result, Osteopontin is thought to have a role in a variety of physiological and pathological processes. Because of its putative regulatory activities on motility and the extracellular matrix, it is now of significant interest in abnormal cell (cancer), renal illness, and idiopathic pulmonary fibrosis. Therefore, we considered the Osteopontin in this study.

2.2.5 The kidney disease test

Symptoms of CKD are usually non-specific. Table 2.3 provides the basic symptoms that are associated with CKD signs. The Clinical signs of CKD are presented on in the natural history of kidney disease. Rarely, it becomes lately to delay the treatment time to prepare for dialysis. The most typical test, however, of renal function using plasma creatinine, is used in the hospital inpatient for investigations during many surgery or hospital clinic outpatient episodes. Oppositely, the chronic obstructive can be found through walking or coughing. In which, there is small chance that it is quantifiable about CKD severity without blood or urine test. Therefore, the discovery of kidney problems can help identifying the CKD, such as the hematuria, proteinuria, structural of kidney, or deficiency kidney function (Rossi *et al.* 2014, Zager *et al.* 2020).

Urine test represents the basic check for the presence and severity of CKD. Testing the urine during the menstrual period in women, and within 2–3. Fresh ‘mid-stream’ urine is most recommended test to reduce accidental contamination with colling at temperatures from +2 to +8°C. Changes in urine color can appear that can identify the CKD severity. Some chemical characteristics of the urine to help detect the CKD can be identified by using dipsticks like urine pH, haemoglobin, glucose, protein, leucocyte esterase, nitrites, and ketones (Powe and Boulware 2009, Hedayati *et al.* 2009, Horio *et al.* 2010, Volckaert *et al.* 2016).

However, the microscopic hematuria can help to identify the CKD. In healthy people, for instance, the red blood cells cannot be found in the urine in more than 95% of patients. Huge amounts of red blood cells change the urine color to pink or red. It is glimpsed that in 3% to 6% of the normal population with 5% to 10% of those relatives of CKD patients who undergo screening for potential kidney donation (Volckaert *et al.* 2016). Finally, other techniques can be applied in order to detect the CKD, such as Microalbuminuria and Proteinuria P, Tests of kidney function, renal imaging, and renal biopsy.

Table 2.3 The basic signs and symptoms of CKD (Yang *et al.* 2016, Mendley *et al.* 2019)

Symptoms	Signs
Tiredness	Pallor
Anorexia	Leukonychia
Nausea and vomiting	Peripheral oedema
Itching	Pleural effusion
Nocturia, frequency, and oliguria	Pulmonary oedema
Hematuria	Raised blood pressure

2.2.6 Serum albumin

A serum albumin test can be considered as simple blood test to measure the amount of albumin in your blood. This can assist the doctors to detect the CKD appearance. A surgery, being burnt, or having an open wound, for example, all enhance the odds of a low albumin level being discovered. If none of these were used and the patient's serum albumin level was abnormal, it's an indication that the kidneys aren't working properly and the patient may have CKD.

The underlying causes that contribute to the start and progression of CKD are unknown, although they include advanced age. Low blood albumin content is an underappreciated risk factor for renal function decrease in general. Decreases in serum albumin content have been linked to cardiovascular illness, and CKD, according to research. The investigations also discovered independently and linked to a greater risk of renal function loss, but numerous inflammatory indicators were not. They also showed that serum albumin concentrations constitute a critical component of a multi-marker prediction model for end-stage CKD development. In which serum albumin levels are used to predict the loss of kidney function and the presence of known liver disease (Fox *et al.* 2004, Lang *et al.* 2013).

The bromocresol green technique is used to determine the content of serum albumin in blood. The blood albumin concentrations are analyzed as a continuous variable per standard deviation and as a categorical variable split into quartiles (> 4.21, 4.01 - 4.21, 3.81 - 4.00, 3.80). The urine albumin to creatinine ratio (ACR) can then be dichotomized at 30 mg/g against lower values, and the urine ACR divided into quartiles. Additionally, there are secondary indications. When the continuous variables are log transformed, the tumor may be found. In general, the levels are associated with seniors' function and risk factors (Goldwasser 1997, Keller *et al.* 2007, Tangri 2011).

2.2.7 S. Electrolyte

Electrolytes are positively charged ions that may be found inside cells and extracellular fluids including intestinal fluid, blood, and plasma. The measuring check for electrolytes is composed of the sodium, potassium, chloride, and bicarbonate. The renal, endocrine, and acid-base functions are all evaluated using these ions. They are components of renal function as well as metabolic biochemistry.

Sodium is largely responsible for balancing the osmotic pressure. High blood sodium levels are observed in people who are dehydrated as a result of diarrhea or vomiting. Decrease of sodium level is a result of low water amount in the body. However, the potassium is a major component in heart function. In this, excessive much potassium content in the blood is produced by poor renal function and might result in irregular and deadly heart rhythms. Whereas the low level of potassium suggests a loss from ingestion of K+ reducing medications, high urination or vomiting. Moreover, the low amount may result in irregular cardiac rhythms. The buffer in the body, serum bicarbonate, can help to maintain a healthy blood pH. Normal blood pH is important for human to stay alive (Cohn 2000, Alebiosu and Ayodele 2005, Mangin and Aussie 2005, Erik *et al.* 2007).

Urea, on the other hand, is a waste product arises from the protein metabolism. It is located in the liver and carried through the blood to the kidneys where it is eliminated. Kidney dysfunction or poor blood circulation to the kidneys can be caused by high blood urea nitrogen levels. Creatinine is a waste product that occurs when muscle consumes energy sources. It travels to the kidneys through the bloodstream. Kidney dysfunction can be caused by a high amount of it. As a result, an electrolyte disorder is defined as an imbalance of certain ionized salts in the blood. These include the bicarbonate, calcium, chloride, magnesium, phosphate, potassium, and sodium. Electrolyte is the ionized molecules generated inside the blood, tissues, and cells. These molecules might be positive, i.e., cation, or negative, i.e., anions. A patient long-term prognosis depends on the underlying effect of the electrolyte imbalance. If it is treated correctly, electrolyte balance in and can be rectified. Some electrolyte imbalances have few to no symptoms

and resolve rapidly if they are minor. It can cause renal impairment ranging from moderate to severe, leading to CKD. Furthermore, CKD develops over time as the nephrons are gradually destroyed (Arije *et al.* 1995, Giovanni *et al.* 2003, Moritz and Ayus 2004). As a result, it is critical to test for electrolytes in the body on a regular basis in order to avoid CKD.

2.2.8 S. Calcium

Calcium is a mineral that has an impact on the human body's health. They help to create strong bones and teeth, as well as cell and nerve function, when combined with phosphate. Both phosphate and calcium are maintained at appropriate levels by the human kidneys and parathyroid glands. Vitamin D is activated by the kidneys and helps to maintain calcium balance. They also regulate how much phosphate is absorbed from meals which regulates phosphate levels in the blood by raising or lowering them (Navaneethan *et al.* 2009, Qassem *et al.* 2013).

If a kidney illness is discovered, the body's calcium and phosphate levels will not return to normal. They are unable to eliminate the extra phosphate from the body once they have reached CKD. Due to a high phosphate level, kidney illness might cause an increase in parathyroid hormone production (See Figure 2.5). Because phosphate binds to calcium, this connection can lead to a drop in calcium levels, which can weaken the bones. It can also lead to skin sores and joint tumors. Figure 2.5 shows a summary of calcium and phosphate balance (Koppe *et al.* 2019).

Figure 2.5 Flowchart of the calcium and phosphate balance of CKD (Ketteler and Biggar 2013)

Phosphate levels can be controlled by reducing the quantity of phosphate in the meals. It is difficult to follow a phosphate-free diet since phosphate is found in so many foods. Furthermore, some meals rich in phosphate must be avoided. For further information, it is suggested that you consult with an Accredited Practicing Dietitian.

3. MATERIALS AND METHODS

3.1 Instruments Used in Analysis

3.1.1 Spectrophotometry medical laboratory device

Figure 3.1 Shows the Spectrophotometry medical laboratory device that used in the present study.

Figure 3.1 Spectrophotometry medical laboratory device–Biometrieux (France), SN (IVD5203427)-Model Mini VIDAS

3.1.2 Centrifuge medical laboratory device

Figure 3.2 Shows the Centrifuge medical laboratory device that used in the present study.

Figure 3.2 Centrifuge medical laboratory device, Japan (CC Med), SN: K21418

3.1.3 Automatic pipette

Figure 3.3 shows the Automatic pipette (medical laboratory device) that used in the present study.

Figure 3.3 Automatic pipette device

3.1.4 Elisa-reader device

Figure 3.4 Shows the Elisa- Reader device type (CUT 4062), it is one of the medical laboratory device that used in the present study.

Figure 3.4 Elisa- Reader device type (CUT 4062), SN (A120050), Germany production

3.1.5 Elisa-washer devicemicroplate washer for microplate reader versamax

Figure 3.5 shows the elisa microplate washer & microplate washer for microplate reader versamax (Model MSLER02), it is one of the medical laboratory device that used in the present study.

Figure 3.5 MSLER02 elisa microplate washer & microplate washer for microplate reader versamax

3.1.6 Laboratory water bath, it is laboratory equipment made from a container filled with heated water

Figure 3.6 shows the laboratory water bath, it is one of the medical laboratory device that used in the present study.

Figure 3.6 Laboratory water bath, A water bath operating at 72°C

3.1.7 Refrigerator laboratory device

Figure 3.7 shows the laboratory refrigerator (cooling device model POL-EKO) that used in the present study, it is dedicated for saving samples with limited conditions.

Figure 3.7 Laboratory refrigerator model (POL-EKO)

3.1.8 Kits used in tests analysis

3.1.8.1 Human osteopontin elisa kit (BMS2066 and BMS2066TEN)

Bone Sialoprotein I (BSP-1), Early T-lymphocyte activation (ETA-1) and secreted phosphoprotein 1 are all names for osteopontin (OPN) (SPP1). OPN was discovered as a SIBLING glycoprotein in osteoblasts. It is an organic component of the bone since it is an extracellular structural protein. It is found on chromosome's long arm, consists in total of 300 amino acids and is rich in acidic residues (aspartic or glutamic acid) which lead to a high negative charge. The protein is highly phosphorylated and glycosylated. The nascent protein has a size of 33 kDa; after posĴranslatonal modificat'ons its molecular weight increases to 44 kDa. OPN is expressed by various tissues, such as bone, teeth, kidney, endometrial and epithelial tissues, but is also found in macrophages, d'fferent types of tumors, T cells and smooth muscle cells. The protein is involved in bone resporption, immune function, angiogenesis, cell survival, and wound repair and cancer biology. OPN has biological functions in bone remodeling and in immune functions, respectively chemotaxis, cell activation and apoptosis. During bone remodeling the osteoclasts remove the old bone which is followed by bone forming through osteoclasts. OPN is able to 'nfluence bone homeostasis by promoting d'fferent'at'on of osteoclasts or by enhancing osteoclast activity. It protects again apoptosis and induce survival and proliferation in several cell types. OPN is also associated with tumor genesis.

Principles of the test

An anti-human osteopontin coated antibody is adsorbed in microwells. Antibodies adsorbed to the microwells create a complex with human osteopontin present in the sample or standard. Unbound biological components are rinsed away after incubation, and a biotin-conjugated anti-human Osteopontin antibody is added. The human Osteopontin captured by the first antibody is recognized by this antibody. After incubation, the unbound biotin-conjugated anti-human osteopontin antibody is removed with a wash step. The biotin-conjugated anti-human Osteopontin antibody interacts with

streptavidin HRP, which is added to the reaction. Unbound Streptavidin-HRP is removed after incubation with a wash step, and the wells are filled with a substrate solution reactive with HRP. The quantity of human Osteopontin contained in the sample or standard determines the development of a colored product. With the addition of acid, the reaction is completed, and the absorbance at 450 nm is measured. Seven human osteopontin standard dilutions were used to create a standard curve, and the concentration of human osteopontin was determined.

Reagents

- 1 Aluminum pouch containing a Microwell Plate (12 strips with 8 wells each) coated with human Osteopontin monoclonal antibody.
- Biotin-conjugated anti-human Osteopontin polyclonal antibody, 1 vial (120L).
- 1 Vial Streptavidin-HRP (150mL).
- 2 vials lyophilized human Osteopontin Standard, 60 ng/mL following reconstitution.
- Sample Diluent: 1 bole (12mL).
- 1 Vial (5mL) Assay Buffer Concentrate 20x (PBS containing 1% Tween TM 20, 10% BSA).
- 1 Bole (50 mL) Wash Buffer Concentrate 20x (PBS with 1% Tween TM 20).
- 1 Substrate Solution (15mL) vial (Tetramethyl-benzidine).
- 1 Stop Solution (15mL) vial (1M Phosphoric acid).
- A total of six adhesive films.

3.1.8.2 Human netrin 1 (ntn1) elisa kit (catalog number CSB E11899h)

Principle of the assay

This assay use the quantitative sandwich enzyme immunoassay technique. A pre-coated microplate with an antibody specific for Ntn1 has been used. Pipette standards and

samples into the wells, and any Ntn1 present is bound by the immobilized antibody. After removing any unattached compounds, the wells are incubated with an antibody specific for Ntn1 conjugated to biotin. Following thorough washing of the wells, avidin conjugated Horseradish Peroxidase (HRP) is added. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells, and color develops in proportion to the amount of Ntn1 bound in the initial step. The development of color is halted and the color intensity is determined.

31.25 pg/mL-2000 pg/mL Detection Range

Sensitivity

Ntn1 in humans has a minimum detectable concentration of less than 7.81 pg. The Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be distinguished from zero in this test. The average O value was determined. D value obtained by multiplying twenty zero standard replicates by their three standard deviations.

Specificity

For the detection of human Ntn1, this test has a high sensitivity and excellent specificity. There was no evidence of substantial cross-reactivity or interference between human Ntn1 and analogues. Note: Due to limitations in our present abilities and knowledge, we were not able to complete the detection of cross reactivity between human Ntn1 and all analogues, thus cross reaction may still occur.

3.1.8.3 Materials provided

Table 3.1 shows the materials and it's quantity that used in the present study.

Table 3.1 Materials provided and it's quantity

Reagents	Quantity
Assay plate	1(96wells)
Standard (Freeze dried)	2
Biotin-antibody (100 x concentrate)	1 x 120 µL
HRP-avidin (100 x concentrate)	1 x 120 µL
Biotin-antibody Diluent	1 x 15 mL
HRP-avidin Diluent	1 x 15 mL
Sample Diluent	1 x 50 mL
Wash Buffer (25 x concentrate)	1 x 20 mL
TMB Substrate	1 x 10 mL
Stop Solution	1 x 10 mL
1 N HCI	1 x 10 mL
1.2 N NaOH/0.5 M HEPES	1 x 10 mL
Adhesive Strip (For 96 wells)	4
Instruction manual	1

3.1.9 Sample collection and storage

Serum allow samples that collected from patient to clot most probably 2 hours at room temperature, or sometime through 24 hours at 4°C before centrifuging in a serum separator tube for 15 minutes at 1000g (SST). Remove serum and test as soon as possible, or aliquot and store samples at -20°C or -80°C. Freeze-thaw cycles should not be repeated. Plasma As an anticoagulant, use EDTA or heparin to collect plasma. Within 30 minutes after collection, centrifuge for fifteen min at 1000 x g, 2 - 8°C. Immediately perform the Assay, or aliquot and store samples for later use at -20°C or -80°C. Repeated freeze-thaw cycles should be avoided. Before the test, centrifuge the material again once it has thawed.

Reagent preparation

1. Before opening the vial of biotin antibody, centrifuge it. A 100-fold dilution is required for biotin antibody. 10 l Biotin antibody + 990 l Biotin antibody DiluentDiluent is a proposed 100-fold dilution.
2. Centrifuge the vial of HRP-avidin before opening it. A 100-fold dilution is required for HRP-avidin. 10 l HRP-avidin + 990 l HRP-avidin DiluentDiluent is a proposed 100-fold dilution.
3. Wash Buffer- If the concentrate has crystallized, bring it to room temperature and gently mix until the crystals have dissolved. Dilute 20mL of Wash Buffer Concentrate (25x) with deionized or distilled water to create 500mL of Wash Buffer (1x).
4. Standard Centrifuge the standard vial at 6000-10000rpm for 30 seconds. Reconstitute the Standard with 1.0mL of Sample Diluent Diluent. Diluents other than water should not be utilized. A stock solution with a concentration of 2000 pg/mL is obtained using this method. Allow a minimum of 15 minutes for the standard to rest with gentle agitation before making dilutions to ensure complete reconstitution. Fill each tube with 250 of Sample Diluent (S0-S6). To make a 2-fold dilution series, start with the stock solution (below). Before moving on to the next transfer, carefully mix each tube. The high standard (2000pg/mL) is the undiluted Standard. The zero standard is sample diluent (0 pg/mL).

3.1.10 Assay procedure

Before using, bring all reagents and samples to room temperature. Before the test, centrifuge the sample once it has thawed again. It is recommended that all samples and standards be examined twice.

1. As indicated in the preceding sections, prepare all reagents, working standards, and samples.
2. Determine the number of wells to be utilized using the Assay Layout Sheet, then return any remaining wells and desiccant to the pouch, seal the bag, and store unused wells at 4°C.

3. Pour 100 l of standard solution and 100 l of sample solution into each well. Cover with the sticky strip that comes with it. At 37°C, incubate for 2 hours. To keep track of the standards and samples analyzed, a plate arrangement is supplied.
4. Drain but do not wash each well's contents.
5. Pour 100 liters of biotin-antibody solution into each well (1x). Remove the adhesive strip and replace it. At 37°C, incubate for 1 hour. (The biotin-antibody (1x) image may appear hazy.) Allow the solution to cool to room temperature before gently mixing until everything is well combined.)
6. For a total of three washes, repeat the aspiration and washing of each well twice more. Using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, fill each well halfway with Wash Buffer (200l) and leave aside for 2 minutes. Aspirate or decant any leftover Wash Buffer after the last wash. Clean the plate by inverting it and wiping it clean with clean paper towels.
7. Pour 100 l HRP-avidin into each well to the top (1x). Changing the sticky strip on the microtiter plate At 37°C, incubate for 1 hour.
8. Repeat the procedure five times using the same aspiration/wash approach as in step 6.
9. Fill each well with TMB Substrate to a capacity of 90 l. Incubate at 37°C for 15-30 minutes. It is essential to use sun protection.
10. Pour 50 l Stop Solution into each well halfway and softly tap the plate to ensure complete mixing.
11. Using a microplate reader calibrated to 450 nm, determine the optical density of each well within five minutes. Depending on whether wavelength adjustment is available, set either 540 or 570 nm. Subtract the 540 and 570 nm readings from the 450 nm data. Any optical flaws on the plate will be removed during this procedure. 450 nm measurements may be higher and less accurate if they are not corrected.

3.1.11 Blood urea (Bl. Urea) (REF: 321 001)

In proteins, urea is the main product of nitrogen metabolism. The urea cycle produces it in the liver and eliminates it in the kidneys. Protein ingestion, protein catabolism, and kidney function all influence urea levels in the circulation. Renal dysfunction or a

consequence of certain illnesses, such as diabetes, infection, congestive heart failure, and certain liver problems, can cause elevated urea levels. The most common screening test for renal function is the measurement of blood urea nitrogen in combination with serum creatinine.

3.2 Methods

3.2.1 Urease-colorimetric method

Assay principle

The following is the reaction that occurs in the assay system:
In the presence of water and urease, urea is hydrolyzed to generate ammonia and carbon dioxide.

$$Urea + H_2O \xrightarrow{Urease} 2NH_3 + CO_2 \quad (3\text{-}1)$$

3.2.2 Serum creatinine assay kit (colorimetric) ab204537

According to kit provide we were performed it.

3.2.3 Serum uric acid (SUA) Kit (ab65344)

The detection method was colorimetric and according to kit provide we were performed it. By apple spectrophotometry and according to kit provide we were performed it.

3.2.4 Total serum bilirubin (TSB)

Figure 3.8 shows the total serum bilirubin (TSB) that used in the present study.

Figure 3.8 Bilirubin testing equipment device and test kits

3.2.5 Serum Albumin (S. ALB) Assay Kit (Colorimetric) (ab235628)

According to kit provide we were performed it.

3.2.6 Serum K (S. K), serum Na (S. Na) and serum Calcium (S. Ca)

The test was performed according to the instrument data provided by the producing company.

4. RESULTS AND DISCUSSION

4.1 Chronic Kidney Disease Patients Analysis

The present study included 110 people (male), their ages between 19 -76 years for analysis. The number of the selected person was set into two groups, the first group represented (Group A) which included 45 people (man), while the second group represented (Group B) which involved 65 people (man), Group B represented patients were suffering from CKD (See Table 4.1).

Table 4.1 Age in patients and HCs

Tests	Group A n=45 Mean ± SD	Group B n= 65 Mean ± SD	Minimum		Maximum		Sig.
			G-A	G-B	G-A	G-B	
Age (Year)	41.5 ± 14.5	53.1 ± 12.1	19	20	73	76	P > 0.05
Bl. Urea (mg/dL)	32.8 ± 7.46	83.6 ± 26.9	19	44	48	189	P > 0.05
S. creatinine	1.27 ± 1.49	2.95 ± 1.20	0.39	0.94	8.94	6.38	P > 0.05
UA (mg/dL)	4.53 ± 1.10	4.49 ± 0.96	2.88	2.09	6.74	6.92	P < 0.05
TSB (mg/dL)	0.73 ± 0.32	2.41 ± 1.16	0.13	0.39	1.48	5.87	P > 0.05
Albumin (mg/dL)	43.1 ± 7.50	26.6 ± 6.94	29	3.20	57	41	P > 0.05
K	4.21 ± 1.0	5.50 ± 1.39	2.57	2.58	594	7.52	P > 0.05
Na	137.8 ± 4.92	128.7 ± 5.90	129	106	148	139	P > 0.05
Ca+	9.17 ± 0.75	7.30 ± 1.39	7.86	4.80	10.8	11.9	P > 0.05
Netrin-1	319.2 ± 63.6	525.6 ± 84.4	205	390	456	699	P > 0.05
OPN	92.5 ± 14.7	227.9 ± 50.1	54	104	129	299	P > 0.05

G-A = that means Group A, G-B = that means Group B, P > 0.05, that means significant, P < 0.05, that means significant

4.1.1 Age

Our results showed the high significant where the results for the mean age of the group A (Control group) and B (Patients group) was (41.5 ± 14.5, 53.1 ± 12.1 year) respectively, as shown in Table 4.1 and Figure 4.1. Where the lowest age was 19 years and the oldest age was 20 years for the control group, while the lowest age for the patient group was 73 years and the oldest age was 76 years. GFR decreases by 10 mL/min for every decade above 40 years at the age of 70. With age The aging process compounded by risk factors make the elderly susceptible to Chronic Kidney Disease (Mallappallil *et al.* 2014), this study agreed with our study.

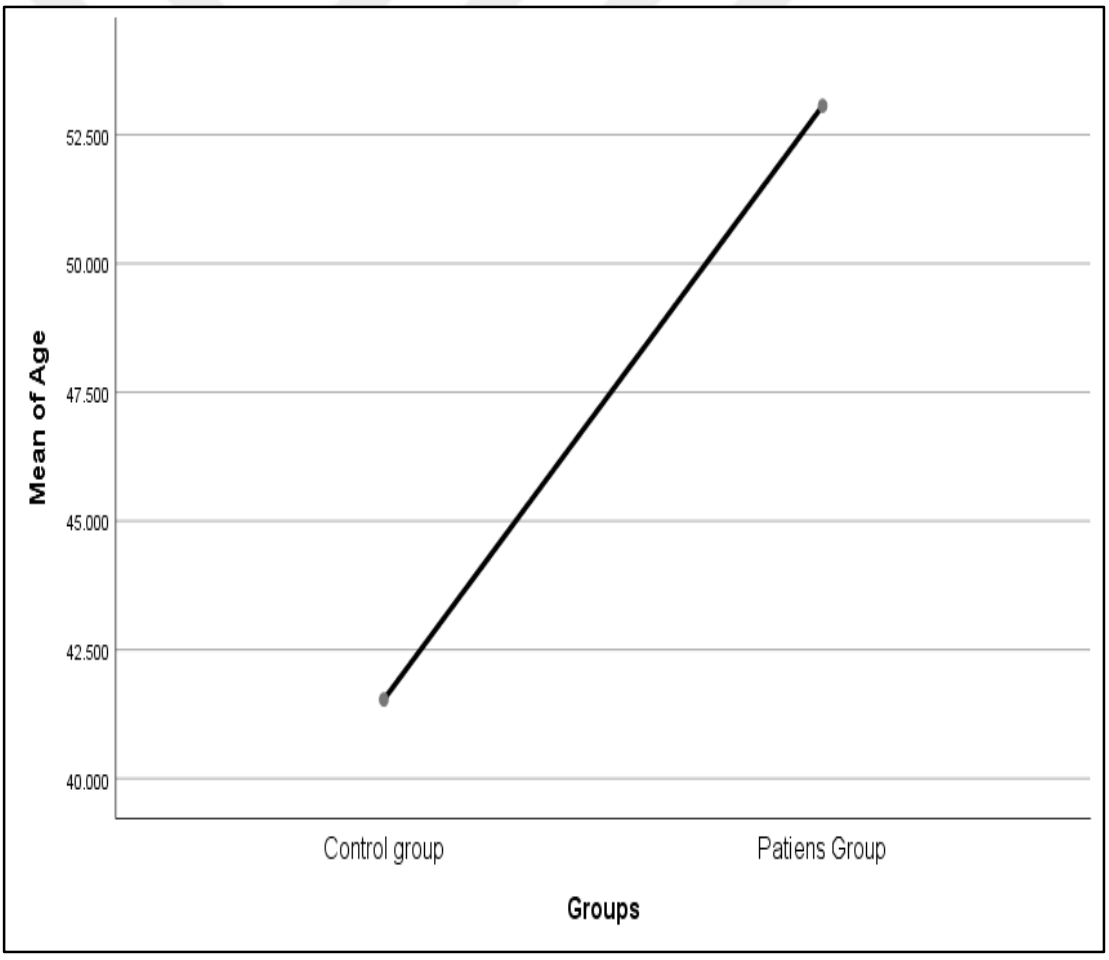

Figure 4.1 The comparison of the mean age for the control group and the patient group

4.1.2 Blood urea and S. creatinine

As shown in Table 4.1 and Figure 4.2 the mean of Bl. urea (mg/dL) was has a significant difference between group A (32.8 ± 7.46) as compare to the B groups (83.6 ± 26.9), where the lowest result was 19 mg/dL and the oldest result was 44 mg/dL for the control group, while the lowest result for the patient group was 48 mg/dL and the oldest result was 189 mg/dL. Also, in our study was the mean of S. creatinine (mg/dL) was has a significant difference between group A (1.27 ± 1.49) when as compare to B groups (2.95 ± 1.20) as shown in Table 4.1 and Figure 4.3, where the lowest S. creatinine result was 0.39 mg/dL and the oldest S. creatinine result was 0.94 mg/dL for the control group, while the lowest S. creatinine result for the patient group was 8.94 mg/dL and the oldest S. creatinine result was 6.38 mg/dL. With a p-value of 0.001, the median serum and blood urea and creatinine levels were higher in the CKD group compared to the controls group (Pandya *et al.* 2016). The nitrogenous end products of metabolism are urea and creatinine. Both molecules are rather tiny (60 and 113 daltons, respectively). The typical range of urea in blood or serum is 15 to 45 mg/dL, and S. creatinine is 0.7 to 1.3 mg/dL (Safhi 2018).

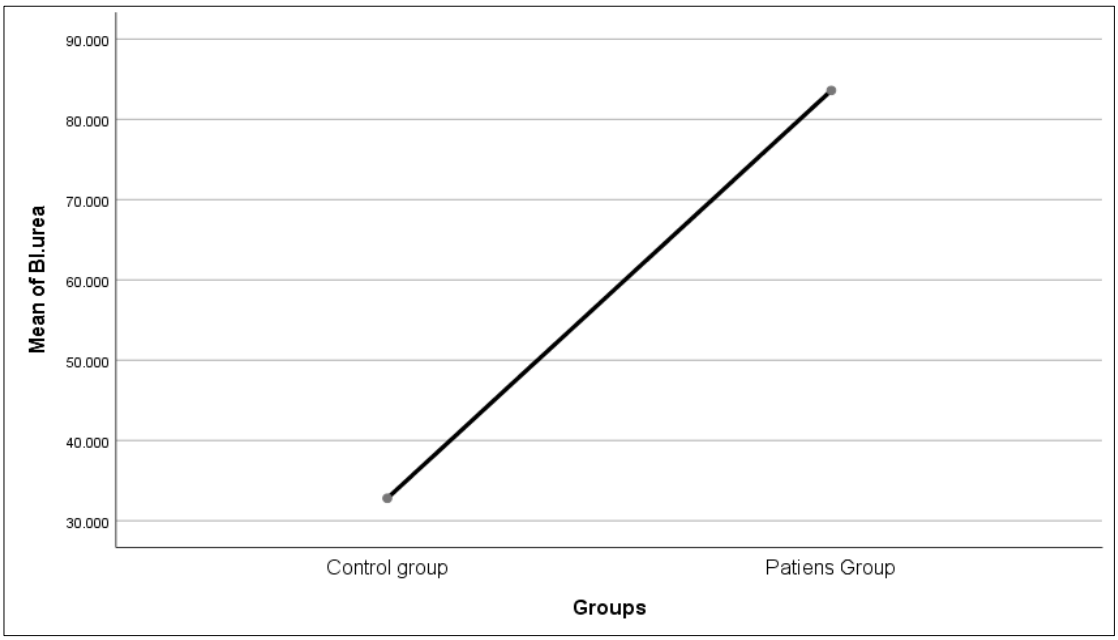

Figure 4.2 The comparison of the mean BI. Urea for the control group and the patient group

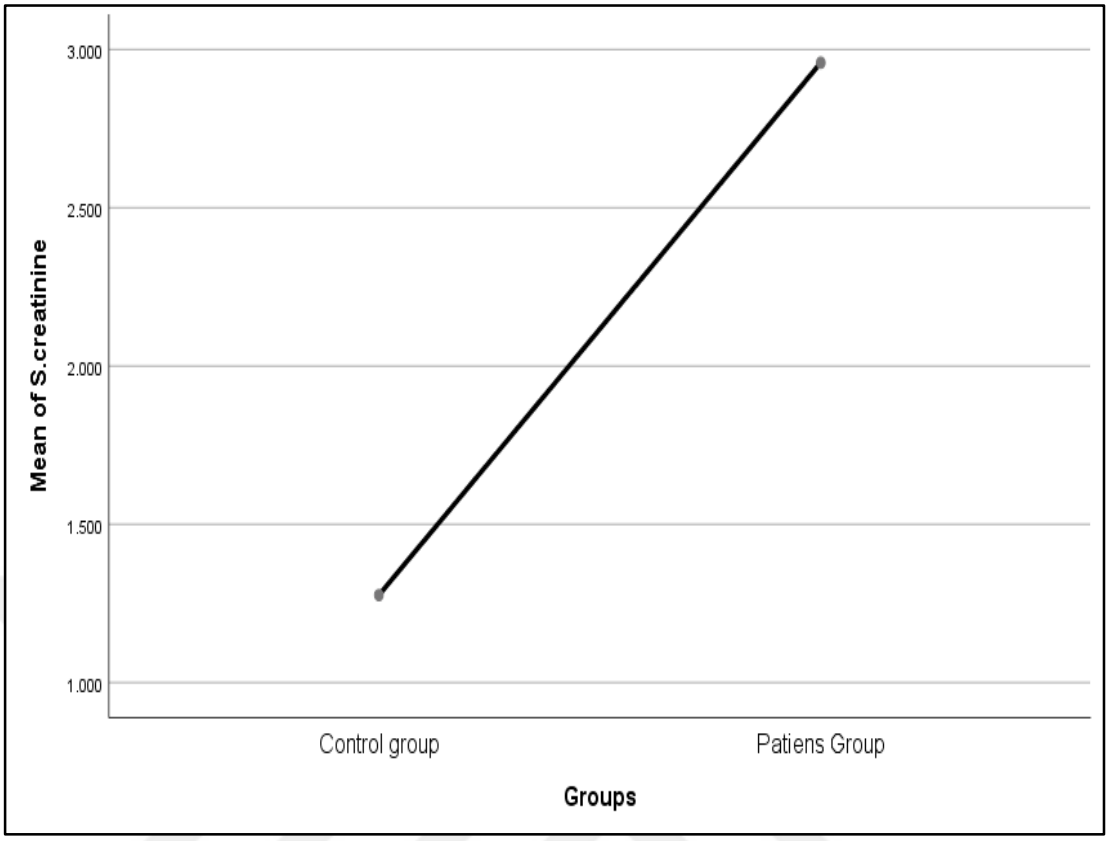

Figure 4.3 The comparison of the mean S. creatinine for the control group and the patient group

4.1.3 Uric acid, TSB and ALB

As shown in Table 4.1 and Figure 4.4, the mean of Uric acid (mg/dL) in patients (group B) was (4.49 ± 0.96), a significant difference when a compared to Control group (4.53 ± 1.10, group A), where the lowest Uric acid result was 2.88 mg/dL and the largest Uric acid result was 2.09 mg/dL for the control group, while the lowest Uric acid result for the patient group was 6.74 mg/dL and the largest Uric acid result was 6.92 mg/dL. Some studies presented so far, derived from experimental, don't identify the correlation between uric acid and CKD like that following from a randomized clinical trial (Bellomo *et al.* 2004), this study agree with the present study.

The mean of TSB (Mg/dL), Albumin (mg/dL) in group B was (2.41 ± 1.16; 26.6 ± 6.94 respectively) which showed a significant difference when a compared to Control group

A (0.73 ± 0.32; 43.1 ± 7.50 respectively), as shown in Table 4.1, Figure 4.5, and Figure 4.6, where the lowest TSB and Albumin result was 0.13 & 29 mg/dL respectively, and the largest TSB and Albumin result was 0.39 & 3.20 mg/dL respectively, for the control group, while the lowest TSB and Albumin result for the patient group was 1.48 & 57 mg/dL respectively, and the largest TSB and Albumin result was 5.57 & 41 mg/dlrespectively. The decreased serum albumin level in patients group while don't change in controls group (healthy person) in Chronic Kidney Disease (Alves *et al.* 2018). In one study, the change in serum bilirubin may predict the progression and followings of Chronic Kidney Disease (Uludag *et al.* 2018).

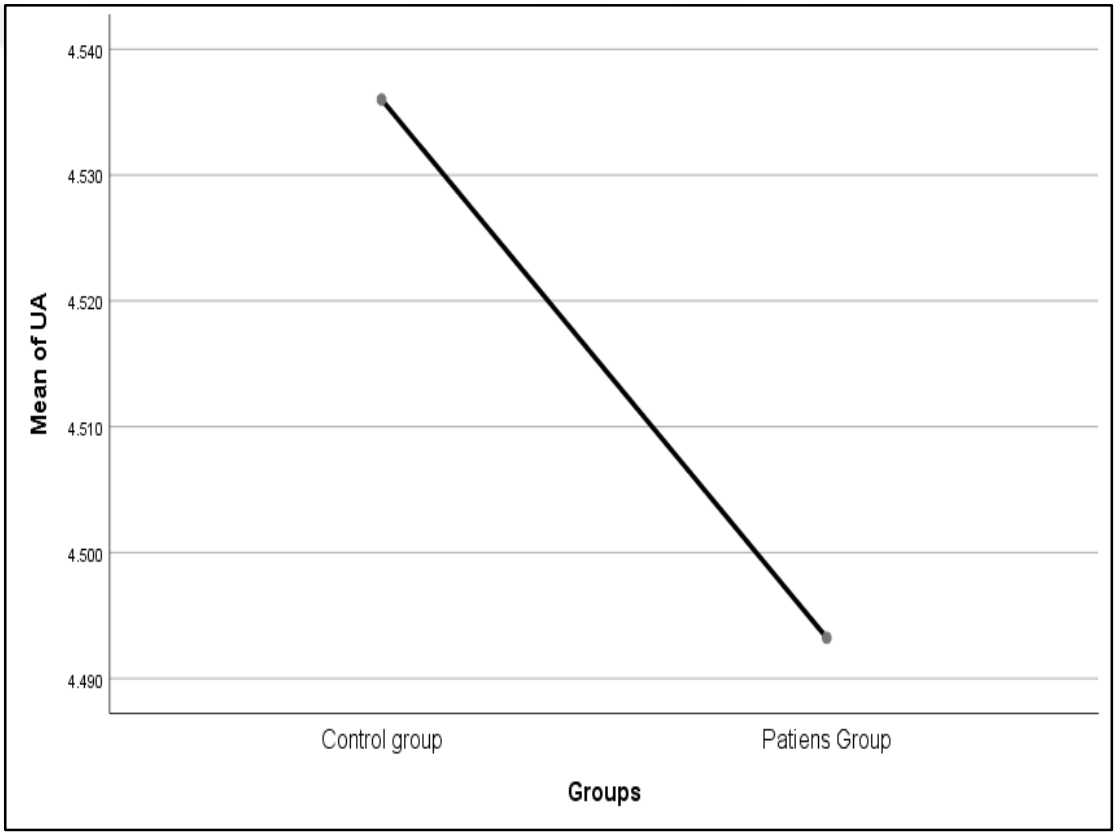

Figure 4.4 The comparison of the mean UA for the control group and the patient group

Figure 4.5 The comparison of the mean TSB for the control group and the patient group

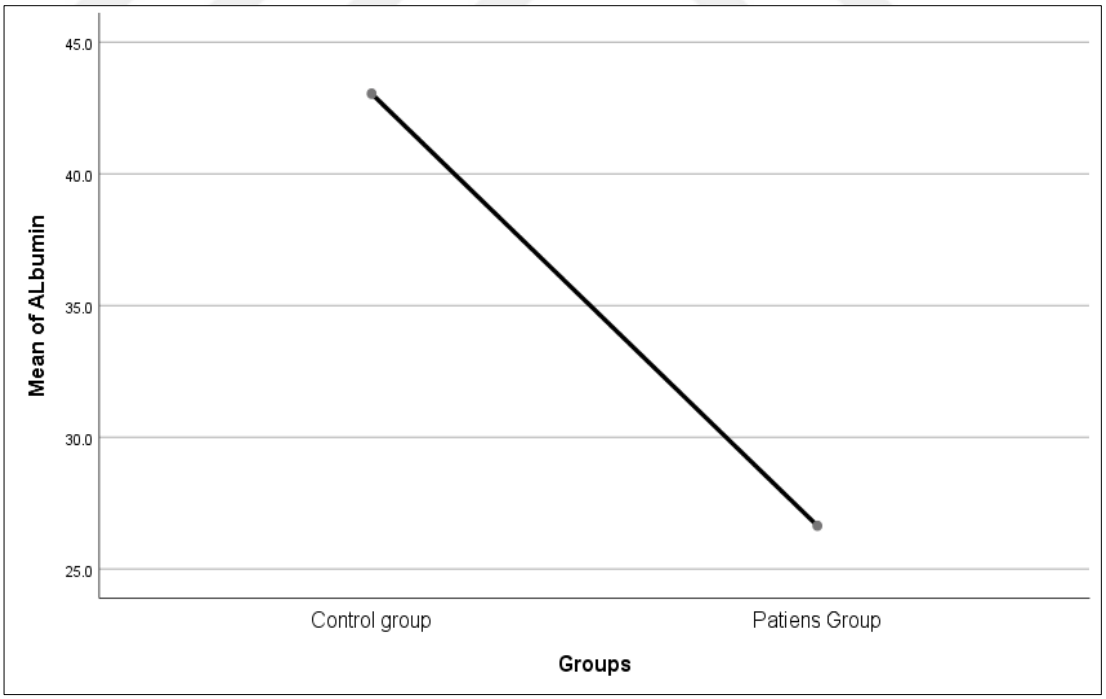

Figure 4.6 The comparison of the mean Albumin for the control group and the patient group

4.1.4 K, Na and Ca

The data of present study indicated that there were significant differences in level of K, Na, and Ca (mg/dL) between group A and B (4.21 ± 1.0; 137.8 ± 4.92; 9.17 ± 0.75) (5.50 ± 1.39; 128.7 ± 5.90; 7.30 ± 1.39) respectively, as shown in Table 4.1 and Figure 4.7, Figure 4.8, and Figure 4.9. Where the lowest K, Na, and Ca result were (2.57, 129, 7.76 mg/dL) respectively, and the largest Uric acid result was 5.94; 148; 10.8 mg/dL respectively, for the control group, while the lowest K, Na, and Ca results for the patient group were 2.58; 106; 4.80 mg/dL respectively, and the largest Uric acid results were 7.52; 139; 11.9 mg/dL respectively. Sodium intake increases the volume of blood inside the blood vessels, and thus raises blood pressure and thus increases pressure in the glomeruli. Overall, the effect is a decrease in kidney function Potassium acts as opposed to sodium, thus increasing sodium excretion. In conclusion, sodium increases in patients with chronic kidney disease, as well as sodium, and also calcium is affected slightly (Koo *et al.* 2018). Average potassium, Na and calcium level was lower in controls group when compared to patients group, except Ca was lower in patients group when compared to control group (Nasir *et al.* 2014). Hence these conclusions are consistent with our study.

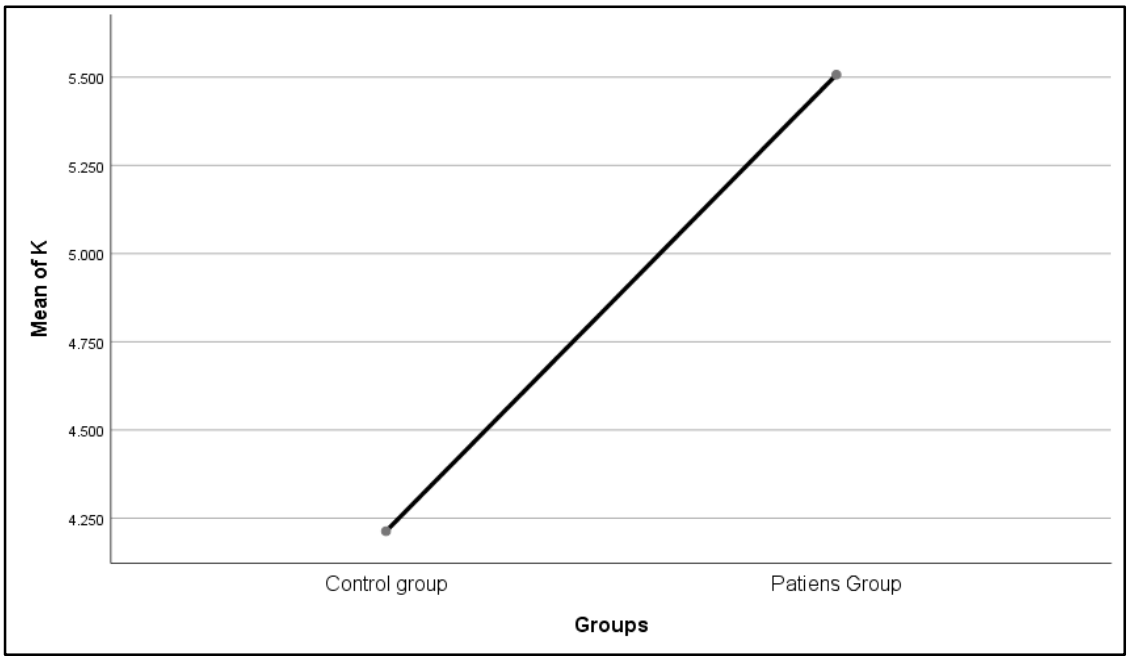

Figure 4.7 The comparison of the mean K for the control group and the patient group

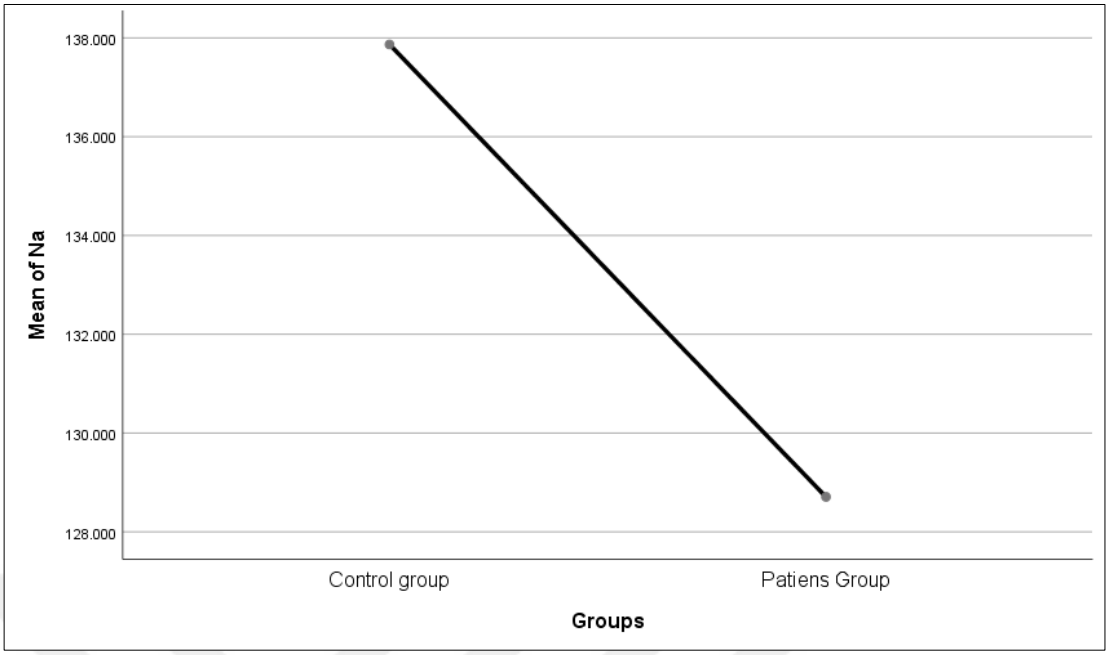

Figure 4.8 The comparison of the mean Na for the control group and the patient group

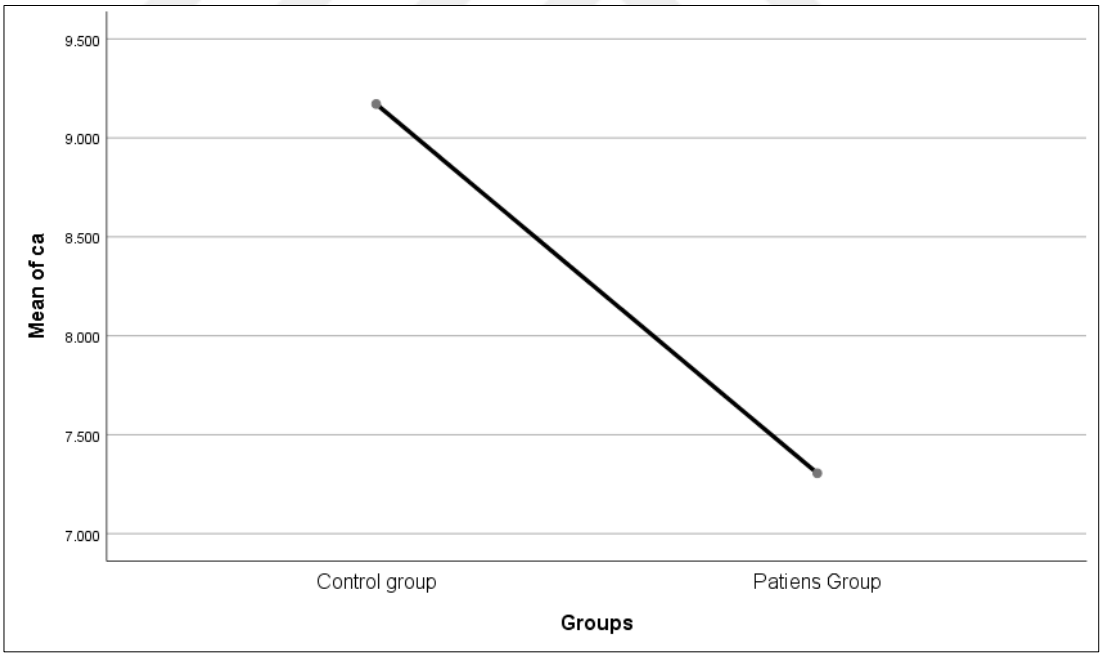

Figure 4.9 The comparison of the mean Ca for the control group and the patient group

4.1.5 Netrin-1

The present results have shown that there is a significant difference between group A (319.2 ± 63.6) compared to group B (525.6 ± 84.4) of the study in the level of Netrin-1, as shown in the Table 4.1 and Figure 4.10. Where the lowest Netrin-1 result was 205 mg/dL and the largest Netrin-1 result was 456 mg/dL for the control group, while the lowest Netrin-1 result for the patient group was 390 mg/dL and the largest Netrin-1 result was 699 mg/dL. Blood netrin-1 level was significantly higher in obese subjects with chronic kidney disease when compared to Controls group (Hacıhamdioğlu *et al.* 2016).

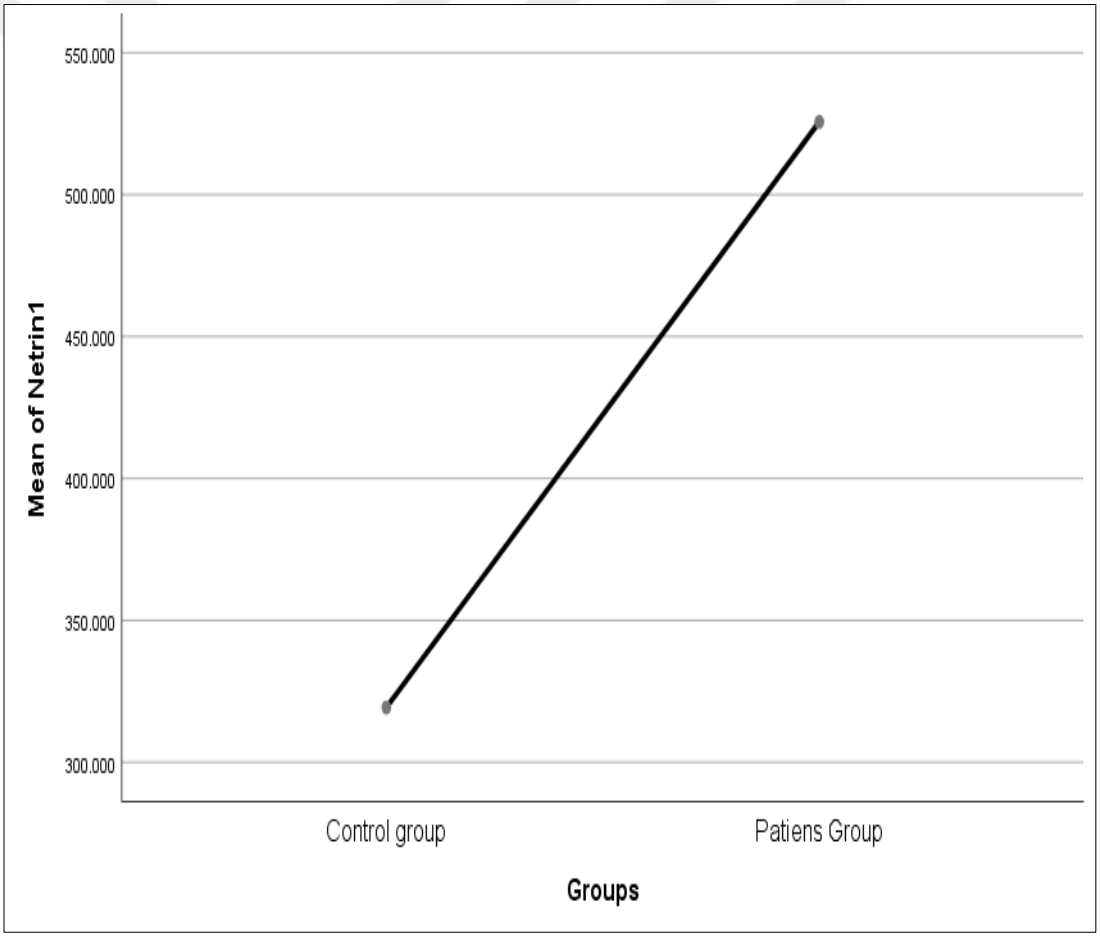

Figure 4.10 The comparison of the mean Netrin-1 for the control group and the patient group

4.1.6 Osteopontin (OPN)

The data of this study refer that significantly higher in OPN level in patients when compared to the control group, as well as observed higher significant difference between group A (92.5 ± 14.7) compared to B group (227.9 ± 50.1) as shown in Table 4.1 and Figure 4.11. Where the lowest OPN result was 54 mg/dL and the largest OPN result was 129 mg/dL for the control group, while the lowest OPN result for the patient group was 104 mg/dL and the largest OPN result was 299 mg/dL. Renal failure is marked by CKD, which usually results in a disruption of bone and mineral metabolism. When compared to a control group, the CKD patients group (n = 92; median: 240.25 ng/mL) had higher plasma OPN levels of n = 92; median: 240.25 ng/mL (Controls group (n = 49; median: 63.30 ng/mL) (Druck *et al.* 2019), this result agreed with our study.

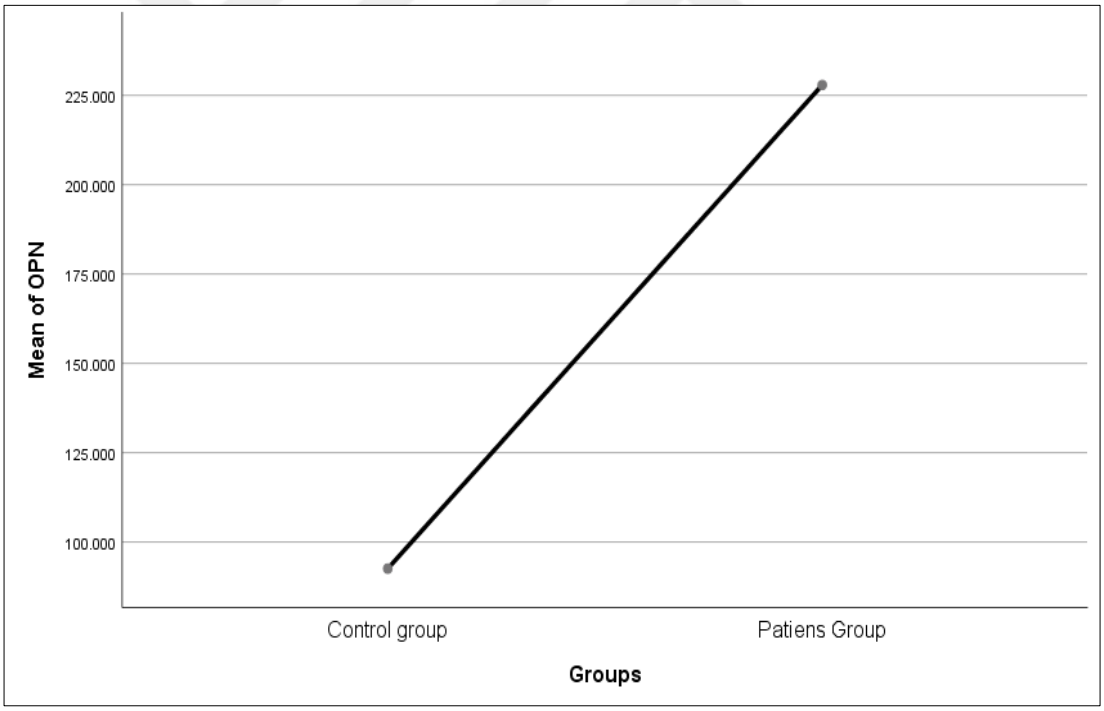

Figure 4.11 The comparison of the mean OPN for the control group and the patient group

5. CONCLUSIONS AND RECOMMENDATIONS

In the present study, it was conducted on patients having from CKD, and the results indicated the importance of age in relation to aging. At the same time, as is known, there was a correlation between the urea test and creatinine.

There was no association between CKD and a gout test at a significance level less than 5 percent. While the results of the study indicated that there are significant differences in the tests (TSB, Albumin) and may be of clinical importance in the follow-up of the disease while receiving treatment.

While our study confirmed the clinical importance of Netrin-1 and OPN, where the study proved their association and their high levels with the change that may occur in patients with CKD.

Which can be useful in the early diagnosis of the stages of disease progression besides the follow-up of the disease during the treatment.

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