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Corin Status and Some Biochemical Parameters among Chronic Kidney Diseased Male Children in Gaza Strip

تقييم مستوى إنزيم الكورين وبعض المعايير البيوكيميائية لدى مرضى الكلى المزمن من الأطفال الذكور في قطاع غزة

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إقـــــرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

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نتيجة الحكم على أطروحة ماجستير

بناءً على موافقة شئون البحث العلمي والدراسات العليا بالجامعة الإسلامية بغزة على تشكيل لجنة الحكم على أطروحة الباحثة/ **هناء محمد عبدالرحيم مهنا** لنيل درجة الماجستير في **كلية *العلوم*** **قسم العلوم الحياتية- تحاليل طبية** وموضوعها:

تقييم مستوى إنزيم الكورين وبعض المعايير البيوكيميائية لدى مرضى الكلى المزمن من الأطفال الذكور في قطاع غزة

Corin Status and Some Biochemical Parameters among Chronic Kidney Diseased Male Children in Gaza Strip

وبعد المناقشة التي تمت اليوم الأربعاء 22 ذو الحجة 1438هـ، الموافق 2017/09/13 الساعة الحادية عشرة صباحاً في قاعة مؤتمرات مبنى اللحيدان، اجتمعت لجنة الحكم على الأطروحة والمكونة من:

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وبعد المداولة أوصت اللجنة بمنح الباحثة درجة الماجستير في كلية *العلوم*/ قسم **العلوم الحياتية - تحاليل طبية**.

واللجنة إذ تمنحها هذه الدرجة فإنها توصيها بتقوى الله ولزوم طاعته وأن يسخر علمها في خدمة دينها ووطنها.

والله ولي التوفيق،،،

عميد البحث العلمي والدراسات العليا

أ.د. مازن اسماعيل هنية

Abstract

Background: Chronic kidney disease (CKD) has been recognized as considerable medical problems for most of the last two centuries**.** This study investigates serum corin levels in CKD patients, revealing higher levels than in normal individuals.

Objective: To assess corin level in patients with CKD and controls from Gaza Strip, and its relationships with some biochemical variables.

Subject and methods: This case-control study comprised 43 cases and 43 controls males below 12 years old. Questionnaire interview was applied. Serum corin, urea, creatinine, uric acid, total protein, albumin, cholesterol, triglyceride, phosphorus, calcium, sodium, potassium and chloride were determined. Data were computer analyzed using SPSS version 22.0.

Results: The mean of cases age was 6.1±3.6 years compared to 6.6±3.3 years of controls (P=0.540). Serum corin was progressively increased showing mean of 1816.3±782.3 pg/ml in cases and mean of 1359.1±442.2 pg/ml in controls. The difference in body mass index (BMI) between cases and controls was highly significant (16.1±2.7 vs. 20.6±3.5 kg/m^2 respectively, P<0.001).The mean of glomerular filtration rate (GFR) was found to be significantly higher in controls compared to cases (143.2±31.9 vs. 57.8±51.4 ml/min/1.73m² respectively, P<0.001). The mean of cholesterol was found to be significantly higher in cases compared to controls (195.4±80.4 vs.159.7±28.9 mg/dl respectively, P=0.007). In addition, triglyceride was significantly higher in cases than in controls (173.1±133.1 vs. 108.4±42.9 mg/dl respectively, P=0.003). There was a significant difference in the calcium level between cases and controls (9.1±1.2 vs. 10.6±0.9 mg/dl respectively, P<0.001). The Pearson correlation test showed positive significant correlation between corin level and urea (r=0.224, P=0.038).) The Pearson correlation test also showed positive correlation with onset CKD and systolic blood pressure but it was not significant (r=0.174, P=0.264 and r=0.029 respectively, P=0.789).

Conclusions: Serum corin was significantly higher in CKD patients compared to controls. Serum corin level has a significant inverse relation with age, height, weight, BMI, total protein, calcium, and GFR. In contrast, Serum corin levels showed significant positive correlations with urea and phosphorus.

Keywords: Chronic kidney disease. Serum corin. Male children. Gaza strip.

ملخص الدراسة

المقدمة: يعتبر مرض الكلى المزمن من أهم المشاكل الطبية على مدى أكثر من قرنيين ماضيين. في هذه الدراسة تم التحقق من مستوي الكورين في مصل الدم لمرضى الكلى المزمن حيث كشفت مستوى أعلي منه مقارنة بالأشخاص الطبيعيين.

الهدف: تقييم مستوى الكورين لدى مرضى الكلى المزمن و كذلك العينات الضابطة وعلاقته ببعض المتغيرات البيوكيميائية.

الطرق والأدوات: منهج الدراسة (مجموعة مرضية – مجموعة الأصحاء)، المجموعة المرضية تحتوي على 43 مريض ذكر و كذلك أيضا مجموعة الأصحاء تحتوى على 43 ذكر جميعهم اقل من 12 سنة. وقد تم إجراء المقابلة الشخصية لتعبئة الاستبيان، وتم قياس مستوى الكورين، اليوريا، الكرياتنين، اليوريك أسيد، البروتين الكلى، الزلال، الكولسترول، الدهون الثلاثية، الفسفور، الكالسيوم، الصوديوم، البوتاسيوم، و الكلورايد، وتم تحليل البيانات والنتائج باستخدام البرنامج الإحصائي SPSS-22.0.

النتائج: كان متوسط الأعمار في المرضى و الأصحاء هو 6.1±3.6 و 6.6±3.3 سنة على التوالي p=0.540، و كان متوسط مستوى الكورين في مرضى الكلى أكثر مقارنة مع الأصحاء و هذه ذات دلالة إحصائية (1816.3±782.3) بيكوجرام/مل للمرضى، مقابل (1359.1±442.2) بيكوجرام/مل للأصحاء، كما أظهرت الدراسة دلالة إحصائية واضحة في فرق كتلة الجسم بين مرضى الكلى (16.1±2.7) مقابل الأصحاء (20.6±3.5) كيلو جرام/متر مربع، p<0.001 .

كما كان متوسط معدل الترشيح في الأصحاء (143.2±31.9) أعلى منه في مرضى الكلى (57.8±51.4) مل/دقيقة/1.73 متر مربع و ذات دلالة إحصائية p<0.001.

و كان متوسط مستوى الكولسترول أعلى في مرضى الكلى منه في الأصحاء (195.4±80.4) للمرضى و(159.7±28.9) للأصحاء مليجرام/ديسيلتر p=0.007 .

بالإضافة أن مستوى الدهون الثلاثية أظهر دلالة إحصائية عالية في مرضى الكلى (173.1±133.1) مقارنة مع الأصحاء (108.4± 42.9) مليجرام/ديسيلتر p=0.003 .

كما أن هنالك دلالة إحصائية في مستوى الكالسيوم بين مرضى الكلى (9.1±1.2) و الأصحاء (10.6±0.9) مليجرام/ديسيلتر على التوالي p<=0.001 .

كما اظهر التحليل الإحصائي علاقة ترابط إيجابية ما بين الكورين و اليوريا (r=0.224, p=0.038).

و ما بين الكورين و بداية حدوث المرض و كذلك ضغط الدم الانقباضي على التوالي r=0.174, p=0.264 . r=0.029, p=0.789

الاستنتاج: يوجد ارتفاع واضح في مستوى الكورين لدي مرضى الكلى المزمن، و لوحظ وجود علاقة عكسية ما بين مستوي الكورين مع العمر، الطول، الوزن، كتلة الجسم، البروتين الكلى، الكالسيوم، و معدل الترشيح. مع وجود علاقة إيجابية بين مستويات الكورين مع اليوريا و الفسفور.

الكلمات المفتاحية: مرضي الكلى المزمن، الكورين، الأطفال الذكور، قطاع غزة.

Dedication

I dedicate this work:

To the memory of my parents

To my brothers for supporting and encouraging me to believe in my self

To my sister for being my guardian during my educational career

To my aunt who always picked me up on time and encouraged me to go on every adventure especially this one

To all my family thanks for always being there to me

To all the Palestinian martyrs and people who have suffered and struggled to have a free Palestine.

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Hanaa M.Muhanna

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List of Abbreviations

ACR: Albumin Creatinine Ratio

ACTH: Adrenocorticotropic Hormone

ADA: American Diabetes Association

ANP: Atrial Natriuretic Peptides

AQP2: Aquaporin-2

ARF: Acute Renal Failure

BMI: Body Mass Index

BNP: Brain Natriuretic Peptides

cGMP: Cyclic Guanosine Monophosphate

CD: Collecting ducts

CKD: Chronic Kidney Disease

CNP: C-type Natriuretic Peptide

CRF: Chronic Renal failure

CVD: Cardiovascular Disease

DBP: Diastolic Blood Pressure

ENaC: Epithelial Sodium Channel

ESRD: End-stage Renal Disease

GC-A: Guanylyl Cyclase Activating

GFR: Glomerular Filtration Rate

GN: Glomerulonephritis

GTP: Guanosine Triphosphate

HF: Heart Failure

HIV: Human Immunodeficiency Virus

NKF: National Kidney Foundation

NKF-KDOQI: National Kidney Foundation Kidney Disease Outcomes Quality Initiative

NPR-A: Natriuretic Peptide Receptor-A

NPR-B: Natriuretic Peptide Receptor-B

NPR-C: Natriuretic Peptide Receptor C

PDE5: Phosphodiesterase 5

PHIC: Palestinian Health Information Center

PKG II: Protein Kinase II cGMP Dependent

PMP: Per Million Populations

PPM: Patient Per Million

RRT: Renal Replacement Therapy

SPB: Systolic Blood Pressure

TAL: Thick Ascending Limb

UAE: Urine Albumin Excretion

USRDS: United State Renal Data System

Chapter 1

Introduction

Chapter 1
Introduction

1.1 Overview

Chronic kidney disease (CKD) is defined as a decreased glomerular filtration rate (GFR), and or increased urinary albumin excretion, and is an increasing public health affair. Prevalence of the disease is estimated to be 8-16% worldwide (Jha, Garcia-Garcia, Iseki, Li, & Naicker, 2013). CKD represents one of the greatest public health confrontations in the 21st century and is associated with considerable cardiovascular morbidity and mortality (Couser, Remuzzi, Mendis & Tonelli, 2011).

CKD has a great effect on healthcare costs and world productivity, particularly in low-income countries where the young people are the most affected population. Early detection of CKD along with a sufficient management of patients is the best strategy to face this disease. Although we assist with raising awareness of health authorities about CKD burden, improving prevention and management programs are still difficult due to the lack of epidemiologic data at the population level (Seck, 2012). While some people face a slow progression of the disease, others rapidly decline, leading to end-stage renal disease (ESRD) and often demand dialysis or transplantation. Predicting how a patient's disease will progress is intractable, making it a challenge for physicians to determine the best course of treatment for each patient (Tuttle, Bakris, Bilous, Chiang, & de Boer, 2014).

Chronic renal failure (CRF) occurs when a kidney is damaged and cannot work efficiently. Kidneys clean waste from the blood, which ejected from the body in urine. If the disease is caught early, damage to the kidney can be slowed, but not stopped totally. CRF is often caused by diseases such as high blood pressure, diabetes, and various kidney diseases (kidney stone, benign prostatic hypertrophy, polycystic kidney disease, drug-induced kidney disease). In some patients, severe infections (eg, hepatitis B or human immunodeficiency virus (HIV) or autoimmune diseases (eg, lupus) can also cause kidney disease (Dirks, Remuzzi, Horton, Schieppati, & Rizvi., 2006).

Atrial and brain natriuretic peptides (ANP and BNP) are peptide hormones produced mostly by cardiomyocytes in the atrium and ventricle, respectively, and are important in maintaining sodium and body fluid homeostasis (Cabiati, Raucci, Liistro, Belcastro, & Prescimone, 2013). In response to volume or pressure overload, ANP and BNP are released from the heart. The biological effects of ANP and BNP are to promote renal sodium and water excretion and decrease systemic vascular resistance, thereby reducing blood volume and pressure (Yeter, Deth, & Kuo, 2013, and Zhou & Wu, 2014).

The human gene for BNP encodes a 134-amino acid pre-pro-BNP precursor, which after removal of the amino terminal 26-amino acid signal peptide gives rise to a 108- amino acid pro-BNP peptide (pro-BNP 1-108). During release into circulation, further processing of pro-$BNP_{1\text{-}108}$ by a proprotein convertase results in the physiologically active 32-amino acid carboxyl-terminal molecule (BNP-32), derived from amino acids 77 to 108, and an inactive amino-terminal fragment (N-terminal-pro-BNP), derived from amino acids 1 to 76 (Dries, 2007).

Proprotein convertases are a family of proteases that split target proproteins, generating mature, biologically active polypeptides. Two proprotein convertases, corin (Ichiki, Huntley, Heublein, Sandberg & McKie, 2011) and furin (Sawada Suda, Yokoyama, Kanda, & Sakamaki, 1997), are considered the most likely pro-BNP processing enzymes. Corin is a type II transmembrane serine protease (Bugge, Antalis & Wu, 2009) that has been identified as the physiological "pro-ANP (Wu F, Yan W, Pan, Morser, & Wu Q., 2002) and pro-BNP (Ichiki, Huntley, & Burnett, 2013) convertase. The enzyme is highly expressed in the heart (Ichiki et al., 2011), primarily in cardiomyocytes (Hooper, Scarman, Clarke, Normyle, & Antalis, 2000), where it uniquely cleaves the inactive natriuretic peptide precursor molecules into biologically active peptide hormones (Wu Q, Xu-Cai YO, Chen S, & Wang W, 2009). Low levels of corin mRNA were reported in some tissues, including the kidney, bone, skin and brain (Fang, Shen, Dong, Liu, & Shi, 2013).

In the kidney, corin expression was existed in the proximal tubule, thick ascending limb (TAL), connecting tubule and collecting duct, Also renal corin expression was significantly reduced in rat models of kidney disease, which may contribute to sodium retention in those animals (Creemers & Khatib, 2007 and Polzin, Kaminski, Kastner, Wang, & Krämer, 2010). In addition, corin may have a native function in the kidney in

regulating sodium excretion (Fang et al., 2013). Consistent with this, impaired sodium excretion and salt-sensitive hypertension were found in corin knockout mice (Wang, Shen, Cui, Jiang, & Chen, 2012).

1.2 Objectives

The general objective of the present study is to assess of serum corin and some biochemical parameters among CKD male children in Gaza Strip.

1.3 Specific Objectives

1. To evaluate (GFR) in patients compared to the controls.
2. To assess serum corin level in patients with CKD and controls.
3. To determine some biochemical parameters in serum including urea, creatinine, and uric acid, in patients and controls.
4. To measure serum total protein, albumin, cholesterol, triglyceride, phosphorus, calcium, sodium, potassium and chloride in patients and controls.
5. To asses relation between corin with previous biochemical parameters.

1.4 Significance

1. CKD among children is a major global public health problem. Rates are expected to increase, largely due to the presence of diabetes and hypertension among children.
2. In the Gaza strip, only one study assessed the diagnostic utility of BNP, corin and furin as biomarkers for cardiovascular complications in type 2 diabetes mellitus patients (Fathy, Abdel Hamid, Zabut, Jamee, & Abu Mustafa, 2015). Thus, this was the first study to assess of serum corin levels among CKD male children in Gaza Strip.
3. Early detection of microalbuminuria delay kidney disease progression before onset of clinical symptoms, thereby leading to increased survival and lower treatment costs.
4. Understanding the role of corin in nephropathy disorders may be helpful in controlling and management of kidney disease.

Chapter 2

Literature Review

Chapter 2
Literature Review

2.1 The Kidneys

2.1.1 Location and Structure

Kidneys are paired retroperitoneal organs located in the posterior part of the abdomen on each side of the vertebral column. In the human, the upper pole of each kidney lies opposite the twelfth thoracic vertebra, and the lower pole lies opposite the third lumbar vertebra. The right kidney is usually slightly more caudal in position. The weight of each kidney ranges from 125 g to 170 g in the adult male and from 115 g to 155 g in the adult female. The human kidney is approximately 11 cm to 12 cm in length, 5.0 cm to 7.5 cm in width, and 2.5 cm to 3.0 cm in thickness. Located on the medial or concave surface of each kidney is a slit, called the hilus, through which the renal pelvis, the renal artery and vein, the lymphatics, and a nerve plexus pass into the sinus of the kidney. The organ is surrounded by a tough fibrous capsule, which is soft and easily removable under normal conditions (Luyckx & Brenner, 2005).

Two special regions can be identified on the cut surface of a bisected kidney: a pale outer region, the cortex, and a darker inner region, the medulla. The medulla is divided into 8 to 18 striated conical masses, the renal pyramids. The base of each pyramid is positioned at the corticomedullary boundary, and the apex extends toward the renal pelvis to form a papilla. On the tip of each papilla are 10 to 25 small openings that represent the distal ends of the collecting ducts (of Bellini). These openings form the area cribrosa. The renal cortex is about 1 cm in thickness, forms a cap over the base of each renal pyramid, and extends downward between the individual pyramids to form the renal columns of Bertin. From the base of the renal pyramid, at the corticomedullary junction, longitudinal elements termed the “medullary rays of Ferrein” extend into the cortex. Despite their name, the medullary rays are actually considered a part of the cortex and are formed by the collecting ducts and the straight segments of the proximal and distal tubules (Tryggvason, Pikkarainen, & Patrakka, 2006).

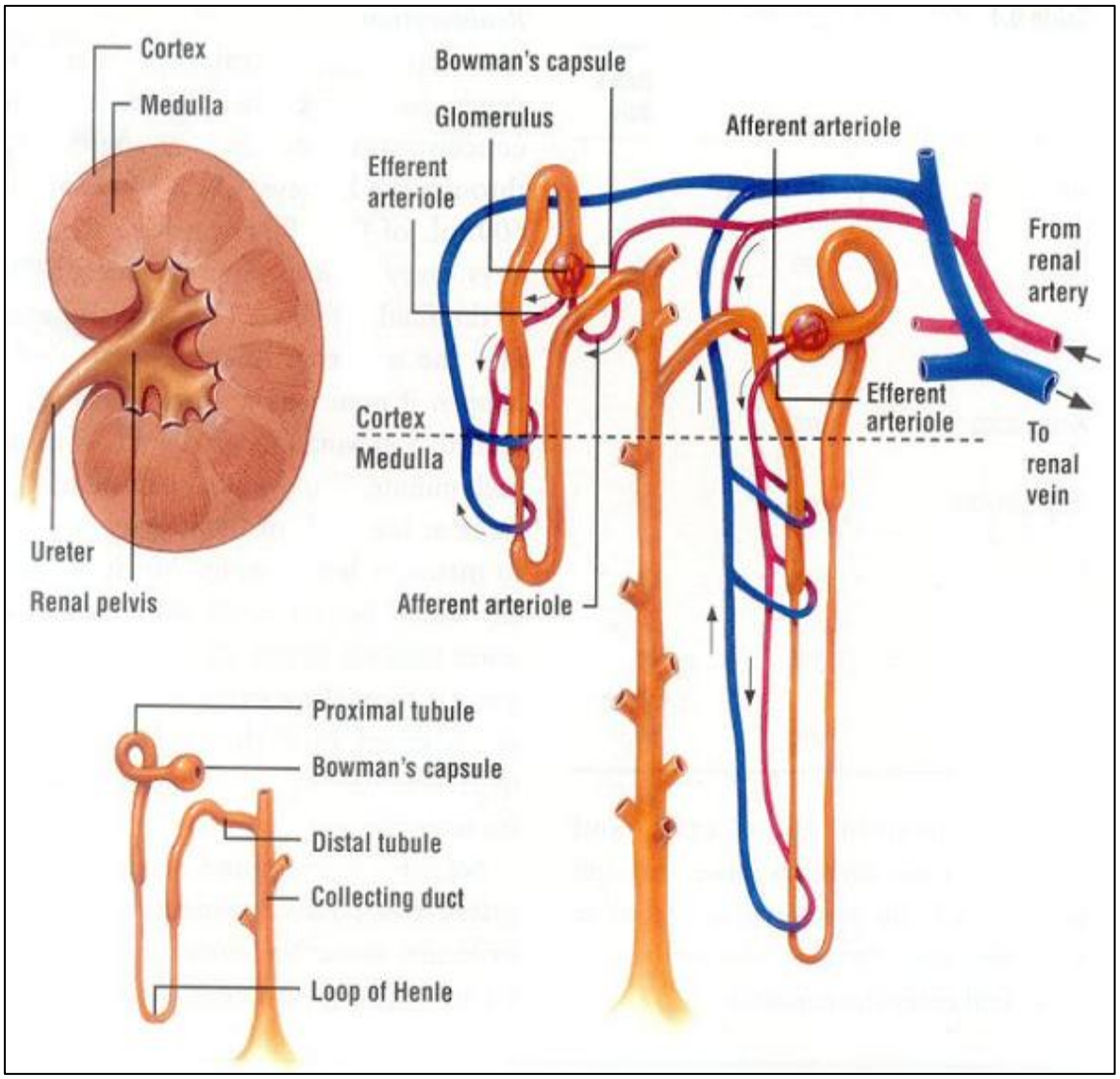

Figure (2.1): Location and structure of the kidney (Tryggvason et al., 2006)

The functional unit of the kidney is the nephron. Each kidney contains approximately one million tiny structures called nephrons (Figure 2.1). Nephrons are responsible for filtration, reabsorption, and secretion that go on in the kidney to form the urine product. The nephron consists of two prime structures, a glomerulus, which is a knot of capillaries, and a renal tubule. The closed end of the renal tubule is enlarged and cup-shaped and completely surrounds the glomerulus. This portion of the renal tubule is called Bowman's capsule. In order from Bowman's capsule, they are the proximal convoluted tubule, loop of Henle, and the distal convoluted tubule. Most of the nephron is located in the cortex, only portion of the loops of Henle dip into the medulla. Urine from many nephrons is collected in the collecting ducts, which transfer

the final urine product into the calyces and pelvis of the kidney (Thibodeau & Patton, 1999).

Every nephron is associated with two capillary beds: The glomerulus and the peritubular capillary bed. The glomerulus is both fed and drained by arterioles. The afferent arteriole is the feeder vessel, and the efferent arteriole receives blood that has passed through the glomerulus. The efferent arteriole then split up to form the peritubular capillary bed, which closely clings to the whole length of the tubule. The peritubular capillaries then drain into an interlobular vein that leaves the cortex (Marieb, 2003).

2.1.2 Role of the Kidneys

The kidneys are two bean-shaped organs that extract waste from blood, balance body fluids, form the urine, and assist in other important functions of the body. The major role of the kidneys is to filter waste products from the blood before converting them into urine. The kidneys also aid in maintain blood pressure, maintain the right levels of chemicals in your body which, in turn, will help heart and muscles function duly, produce the active form of vitamin D that keeps bones healthy, produce a hormone called erythropoietin, which stimulates production of red blood cells (Chand, 2015).

2.1.3 Principles of Renal Pathophysiology

Renal injury can be characterized as either acute or chronic. Each has a distinctive clinical expression.

2.1.3.1 Acute Renal Failure

Acute renal failure (ARF) is known as ″a rapid decline in renal filtration function″. This condition is commonly marked by a high in serum creatinine concentration or azotemia (a rise in blood urea nitrogen concentration) immediately after a kidney injury. Emergency dialysis may be needed until the situation fixed and the kidneys begin functioning again (Agraharkar, 2007).

ARF typically presents with the symptoms of volume overload secondary to impaired urine formation or excretion. The consequent retention of sodium and therefore of water can cause an expansion of the intravascular spaces and extravasation

of fluid into the interstitial space throughout the body. The resulting volume expansion can therefore present as peripheral edema, pulmonary edema, or congestive heart failure. In AR, both acidemia (resulting from failure to excrete or buffer the endogenous metabolic production of acids) and hyperkalemia (resulting from the lack of excretion of dietary potassium) can result in cardiac arrhythmias and sudden death. Acute uremia has a particularly inhibitory effect on platelet function resulting in an increase in the bleeding tendency (Schreiner & Kissane, 1990).

2.1.3.2 Chronic Renal Failure

In CRF the metabolic outcome of uremia are slowly progressive in nature (Glassock, 1987). Chronic acidosis can affect myocardial contractility; contribute to central nervous system toxicity. Water and salt intake persistently overrun excretory capacity, edema formation occurs. Chronic sodium retention can apparent as persistent arterial hypertension. Kidney also fails to convert 25-hydroxyvitamin D to the metabolically active 1, 25-dihydroxyvitamin D, resulting in the disgraceful absorption of calcium from the intestinal tract; secondary hypocalcemia induces secondary hyperparathyroidism with concomitant demineralization and resorption of bone. Reduction of red cell production is the consequence of decreased renal production of the hormone erythropoietin. Increased red blood cell demolition resulting from uremic toxins as well as the mechanical damage to red cell observed in a variety of glomerular disease. Finally, patients with chronic kidney failure had dispirited cellular immunity and humoral immunity (Schreiner et al., 1990).

2.2 Chronic Kidney Disease

2.2.1 Definition of CKD

The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) in the USA defines CKD as ″kidney damage for ≥ 3 months, as assured by kidney biopsy or markers of kidney damage, with or without a decrease in GFR or GFR < 60 mL/min/1.73 m^2 for ≥ 3 months, with or without kidney damage″. Kidney damage is achieved by either kidney biopsy or markers of kidney damage, such as urine abnormalities (proteinuria), blood abnormalities or aberration on imaging studies (Johnson, 2012).

GFR provides a stellar measure of the filtering capacity of the kidneys. A low or decreasing GFR is a good index of CKD. Since the total kidney GFR is equal to the sum of the filtration rates in each of the functioning nephrons, the total GFR can be used as an indicator of functioning renal mass (White, Polkinghorne, Atkins, & Chadban, 2010 and Delanaye, Cavalier, Mariat, Nicolas, & Jean-Marie, 2011). Table 2.1 illustrates normal GFR.

Table (2.1): Normal GFR

Age	Mean GFR±SD (mL/min/1.73 m^2)
1 week (males and females)	**41±15**
2–8 weeks (males and females)	**66±25**
>8 weeks (males and females)	**96±22**
2–12 years (males and females)	**133±27**
13–21 years (males)	**140±30**
13–21 years (females)	**126±22**

*Adopted from NKF, 2002.

A decrease in GFR forgoes kidney failure in all forms of progressive kidney diseases. Monitoring changes in GFR can plan progression of kidney disease. The level of GFR is a strong predictor of the time of onset of kidney failure as well as the risk of the complexity of CKD (National Kidney Foundation (NKF), 2002 and Mungrue Khan, Bisnath, Jaipaul, & Doodhai, 2016). The level of GFR should be estimated from prediction equations that take into account the serum creatinine concentration and some or all of the following variables: age, gender, race, and body size (Table 2.2). The following equations provide useful estimates of GFR:

- In children, the Schwartz and Counahan-Barratt equations
- In adults, the abbreviated Modification of Diet in Renal Disease (MDRD) Study equation and Cockcroft-Gault equations (NKF, 2002).

Table (2.2): Prediction of GFR based on serum creatinine.

Equation Author	Equation
Schwartz	GFR (ml/min/1.73m²) = 0.55×length ⁄Scr
Counahan-Barratt	GFR (ml/min/1.73m²) = 0.43 × length ⁄Scr
Abbreviated MDRD Study	GFR(ml/min/1.73m²)=186×(Scr)×(Age)×(0.742 if female) × (1.210 if black)
Cockcroft-Gault	Ccr (ml/min) = (140 – Age)×Weight ×(0.85 if female)⁄ 72× Scr

Scr: serum creatinine, Ccr: creatinine clearance

Proteinuria not only defines the existence of CKD but also has important implications for diagnosis of the type of kidney disease and is associated with a worse prognosis for both kidney disease advancement and the development of cardiovascular disease (CVD) (Sarnak Levey, & Schoolwerth, 2003). Twenty-four hour or other timed collections were the traditional way to measure urine albumin excretion (UAE) but measuring urine albumin/creatinine ratio (ACR) in a spot collection of morning urine in the fasting state is currently recommended as a simple, quick and relatively accurate way of determining albuminuria (American Diabetes Association (ADA), 2004; Busby & Bakris, 2004). Albuminuria is defined as an ″ACR of 30 mg/g or higher″, with microalbuminuria defined as an ″ACR of 30 to 300 mg/g″, and macroalbuminuria defined as an ″ACR over 300 mg/g″(Table 2.3) (Johnson, 2012).

Table (2.3): Stages of CKD should be based on kidney damage (albuminuria/proteinuria), irrespective of the underlying diagnosis

Kidney damage stage*	Urine albumin/ creatinine ratio (mg/mmol)	24h urine albumin (mg/day)	Urine protein: creatinine ratio (mg/mmol)	24h urine protein (mg/day)
Normoalbuminuria	<2.5 (M) <3.5 (F)	<30	<4 (M) <6 (F)	<50
Microalbuminuria	2.5-25 (M) 3.5-35 (F)	30-300	4-40 (M) 6-60 (F)	50-500
Macroalbuminuria	>25 (M) >35 (F)	>300	>40 (M) >60 (F)	>500

2.2.2 Classification of CKD

CKD has been classified into different stages for the purpose of prevention, early identification of renal damage and institution of preventive measures for progression of the primary damage and appropriate guidelines for instituting management for forbidding of complications in severe CKD (Vijayakumar, Nammalwar, & Prahlad, 2007). NKF classified CKD into 5 stages according to the level of GFR (Table 2.4). For stages 1 and 2, kidney damage was assessed by spot albumin-to-creatinine ratio (NKF, 2002).

Table (2.4): Classification of the stages of chronic kidney disease (Johnson, 2012**)**

Kidney function stage	GFR (mL/min/1.73 m2)	Description
1	90	Normal or increased GFR
2	60-89	Normal or slightly decreased GFR
3A	45-59	Mild-moderate decrease in GFR
3B	30-44	Moderate-severe decrease in GFR
4	15-29	Severe decrease in GFR
5	<15 or on dialysis	End-stage kidney failure

2.2.3 Epidemiology and Etiology of CKD

ESRD is increasing worldwide. Renal replacement therapy (RRT) and kidney transplantation are increasing the load on health systems (Ghonemy, Farag, Soliman, El-okely, & El-hendy, 2016). This condition is particularly serious in developing countries where health resources are inappropriate (Stengel, Billon, van Dijk, Jager, & Dekker, 2003). Worldwide, the number of patients receiving RRT is estimated at more than 1.4 million, with the annual incident rate growing to 8% (Schieppati & Remuzzi, 2005). ESRD has many causes that change from one patient to another. The key risk factors for (CKD) are the increasing age of the population, diabetes mellitus and hypertension and medications, such as the use of analgesics regularly over long durations of time resulting in analgesic nephropathy and kidney injury. Polycystic kidney disease is an example of a hereditary cause of CKD. Diabetes is the largest single cause of ESRD in the United Kingdom, accounting for 30-40% of all cases (Sandra, 2005).

In many Arab countries, obstructive uropathy constitutes a major cause of ESRD (40%). The two most common underlying causes are renal calculi and schistosomiasis. In many developing countries, chronic glomerulonephritis is often caused by infections, infestations, and is a leading cause of CKD (Ulasi, Arodiwe, & Ijoma, 2006). The body

of evidence for other modifiable risk factors such as lifestyle factors is growing as some studies propose that tobacco use is positively associated with CKD (Shankar, Klein R., & Klein BE., 2006). Alcohol has been linked as a cause of kidney damage in some clinical and experimental studies (Schaeffner, Kurth, de Jong, Glynn, & Buring, 2005). Also, obesity seems to be an important-and potentially preventable-risk factor for CRF. (Ejerblad, Fored, Lindblad, Fryzek, & McLaughlin, 2006).

Worldwide, the prevalence of ESRD differs extremely. According to the United States Renal Data System, the highest prevalence was found in Taiwan, with 2447 patients per million (ppm), and the lowest prevalence was in Philippines, at 110 pmp. In the United States, the prevalence was 1811 pmp (United States Renal Data System, (USRDS), 2011). In Europe, the prevalence has increased from 760 pmp in 2004 to 889 pmp in 2008 (Stel, Luijtgaarde, Wanner, & Jager, 2011). In Palestine, Khader, Snouber, Alkhatib, Nazzal, & Dudin, (2013), were reported the prevalence of patients with ESRD on dialysis during the study period was 240.3 per million populations (PMP) and they showed the highest prevalence was seen in Jericho city. There were 57.7% males and 42.4% females in the study. The majority of patients (62.3%) were living in villages, while 28.8% were living in cities and 8.9% were living in refugee camps. Most of the patients (45%) were aged between 45 and 64 years. The vast majority of patients were either diabetic (22.5%) or hypertensive (11.1%) or both at the same time (10.6%). There were a considerable number of patients in whom the cause was undetermined (27.6%). The majority of recorded cases of congenital causes were from the Hebron, Jenin and Tubas districts. The prevalence of ESRD noted in the study was comparable with other regional countries but far below the rate recorded in industrialized countries. In the Palestinian territories, there is a general deficiency of national statistics and surveys, particularly in the public health section. Increased efforts and awareness should be focused on the prevention and treatment of diabetes mellitus and hypertension as they are the major causes of ESRD. There should also be an additional enhancement and implementation of strategies for the registration of data in order to conduct periodic comparisons and analytical studies to improve the management and quality of life of ESRD patients. Most common causes of CRF in Jenin district were diabetes mellitus (33.3%), hypertension (16.7%), and chronic glomerulonephritis (13.1%). Inherited kidney diseases formed an important percentage (17.9%) and included primary

hyperoxaluria (10.7%), Alport's syndrome (5.9%), and adult polycystic kidney disease (1.2%) (Abumwais, 2012).In children there is a wide range of conditions and causes of CKD such as: Intrauterine infections, drugs intake in early pregnancy, genetic kidney disease, and congenital anomalies of kidney, postnatal infections, metabolic diseases, and nephrotoxic drugs (Vijayakumar, 2007 and Fathallah-Shaykh, Flynn, Pierce, Abraham, & Blydt-Hansen, 2015). There are no recent data about the prevalence of ESRD; however, the last statistics were performed by Palestinian Health Information Center (PHIC) in 2005, with the prevalence of renal failure was 4% with an incidence of 10.8 per 100,000. (PHIC, 2005)

2.3 Natriuretic Peptide System

The natriuretic peptide system is firstly an endocrine system that maintains fluid and pressure homeostasis by modulating cardiac and renal function. The physiologic functions of the NP system in healthy humans and in patients with cardiovascular disease (CVD) are not totally understood. Natriuretic peptide levels are elevated in patients with heart failure (HF) and other cardiac diseases; measurement of NPs may be used in the clinical setting to help diagnosis and prognosis. In addition, synthetic natriuretic peptides such as nesiritide are available for use in management of patients with acutely decompensated congestive HF (Silver, 2006 & Cacciapuoti, 2010).

Natriuretic peptide is composed of neurohormones synthesized by the heart, brain and other organs. The heart was not a mechanical pump alone, but also an endocrine organ that had powerful effects on blood circulation. Natriuretic peptides caused both natriuresis and diuresis, and they responded to a volume overload which caused either stretch or pressure on the heart (Arjamaa, 2014). NP plays an important role in regulating oxygen transport both locally and systemically, by causing volume contraction (diuresis, natriuresis and plasma shift) leading to hemoconcentration and an increased oxygen-carrying capacity per unit volume of blood (Arjamaa & Nikinmaa, 2013).

The mammalian natriuretic peptide system consists of some substances: atrial natriuretic peptide (ANP); brain or B-type natriuretic peptide (BNP); C-type natriuretic peptide (CNP) (Van Den Berg, Crijns, Van Veldhuisen, Van Gelder, & De Kam, 1998). Like many peptide hormones, natriuretic peptides are synthesized as prepropeptides.

After the signal peptide is removed, an additional proteolytic cleavage is required to convert the inactive propeptide to an active peptide (Wu et al., 2009).
ANP and BNP are primary for maintaining normal blood volume and electrolyte homeostasis (Fang et al., 2013).

2.3.1 Mechanisms of Synthesis and Release of Natriuretic Peptides

2.3.1.1 Atrial Natriuretic Peptide

ANP is a cyclic 28-amino-acid polypeptide synthesized and secreted at most by the atria in the normal adult heart. It is stored in atrial granules as the C-terminal part of the 126-amino-acid prohormone (proANP) (Heikki, 2003). On secretion, $proANP_{1–126}$ is split by the serine protease corin into an N-terminal fragment of 98 amino acids (NT-$ANP_{1–98}$) and the biologically active $ANP_{99–126}$ in equimolar amounts (Wu et al., 2002).

The measurements of NT-ANP can be used to assess the release of ANP from the heart. NT-ANP has a significantly longer half-life (10 times) in plasma compared with ANP (half-life of 2–5 min) and thus has up to 10–50 times the plasma concentration of ANP. NT-ANP is also more stable under laboratory conditions than ANP. ANP is rapidly removed from the circulation mainly through binding to clearance receptors and hydrolysis by neutral endopeptidase. The plasma levels of ANP are changeable, and its credible measurement requires a laborious extraction step. Because NT-ANP is less variable and has a longer half-life within circulation, NT-ANP appears to be a more delegate marker of prolonged cardiac overload than ANP (Figure 2.2) (Heikki, 2003).

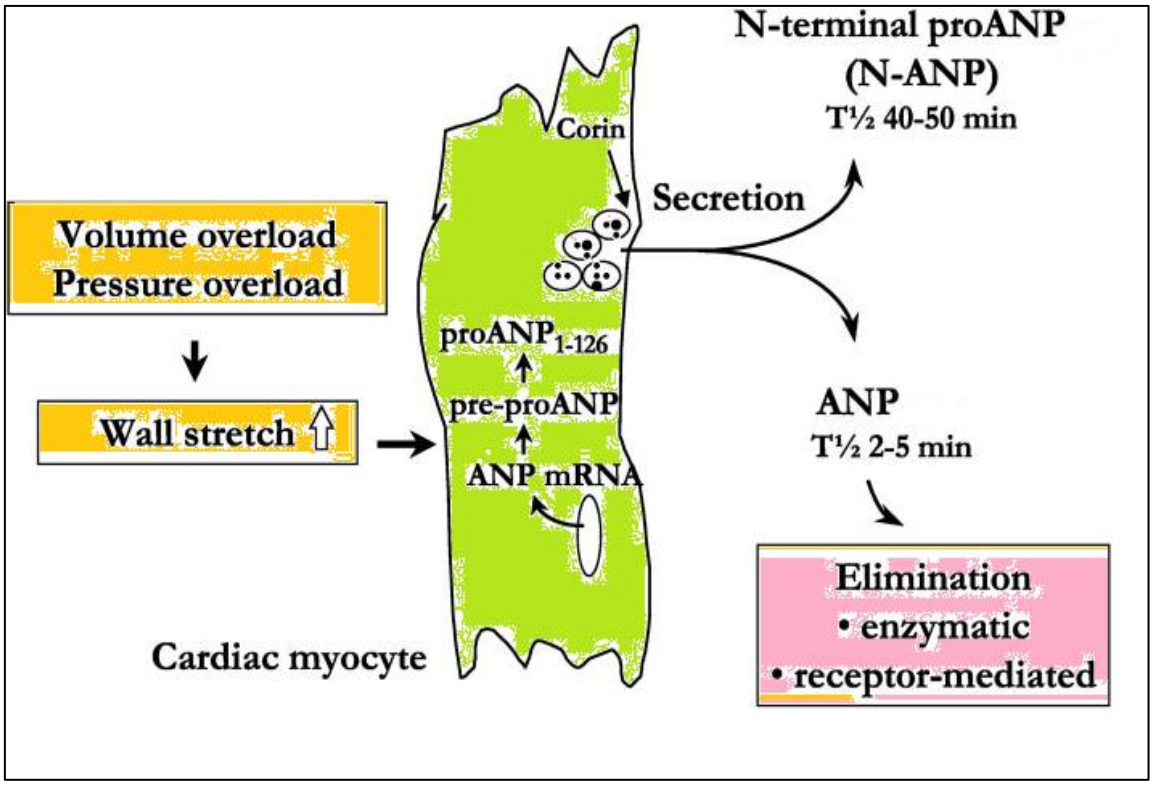

Figure (2.2): Schematic representation of the synthesis and half-life time of the molecular forms of the atrial natriuretic peptide (Heikki, 2003).

2.3.1.2 Brain or B-type Natriuretic Peptide

BNP was originally discovered in porcine brain, where it was thought to be a neurotransmitter, hence its original name, brain natriuretic peptide. Thereafter, it was shown to be 10-fold much more in the heart than in the brain, hence the current term, B-type natriuretic peptide (Christoffersen, Goetze, Bartels, Larsen, & Ribel, 2002).

There appears to be little storage of BNP in the ventricle, which incidentally is the main source. ProBNP is remedied within the human heart to form BNP (consisting of 32 amino acids) with amino acids 77–108 of its 108 amino acid prohormone and an N-terminal proBNP peptide (amino acids 1–76; NT-proBNP) (Figure 2.3). BNP is produced by direct synthesis in response to the degree of ventricular stretch and also upregulated in failing ventricular myocardium. The messenger RNA for proBNP is unstable, so there is active regulation of BNP levels according to ventricular wall tension. Hence, it acts as a reliable biomarker of ventricular dilatation (Witthaut, 2004).

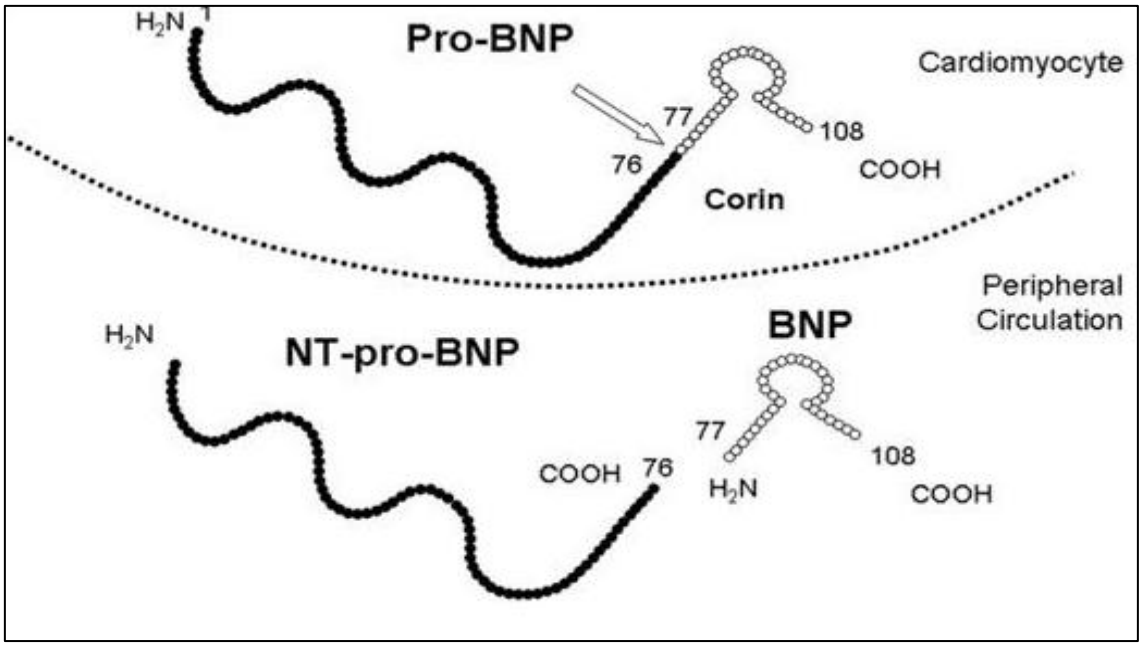

Figure (2.3): Schematic representation of the synthesis and molecular forms of the B-type natriuretic peptide (Troughton & Richards, 2009).

Although BNP and NT-proBNP are synthesized in a 1:1 ratio, their plasma concentrations are different because of their different half-lives in vivo. BNP is cleared at most from the circulation by the natriuretic peptide C receptor and degraded by neutral endopeptidase, whereas NT-proBNP is cleared by the kidneys. Therefore, NT-proBNP concentrations inversely correlate with the GFR and increase with age. The half-life of BNP is only 22 min, whereas the half-life of NT-proBNP is much longer, >120 min with a normal GFR. In vitro, BNP is less stable than NT-proBNP if blood is not collected in plastic tubes containing EDTA as an anticoagulant (Gobinet-Georges,Valli, Filliatre, Dubernet, & Dedeystere, 2000).

BNP is more stable than ANP in plasma (McNairy, Gardetto, Clopton ,Garcia, and Krishnaswamy, 2002) and has a longer half-life (22 min), which may be attributable to its lesser affinity for clearance receptors and neutral endopeptidases (Lang, Motwani, Coutie, & Struthers, 1992). Studies on the stability of NT-BNP in stored plasma are identical to those reported for BNP (and better than ANP) and indicate that the laboratory handling, processing, and storage of NT-BNP can take on without special procedures (Thygesen, Mair, Mueller, Huber, & Weber, 2012).

In the kidney, ANP and BNP exert hemodynamic/glomerular effects which increase sodium and water delivery to the tubule, in combination with inhibitory effects on tubular sodium and water reabsorption leading to remarkable diuresis and natriuresis (Armaly, Assady, & Abassi, 2013).

2.3.1.3 C-type Natriuretic Peptide

CNP is the extreme highly expressed natriuretic peptide in the brain and is found in high concentrations in chondrocytes (Hagiwara, Sakaguchi, Itakura, Yoshimoto, & Furuya, 1994) and cytokine-exposed endothelial cells (Suga, Nakao, Itoh, Komatsu, & Ogawa, 1992). Human proCNP contains 103 residues, and the intracellular endoprotease furin has been shown to process proCNP to 53-amino-acid peptide in vitro (Figure 2.4) (Wu C, Wu F, Pan, Morser, & Wu Q., 2003).

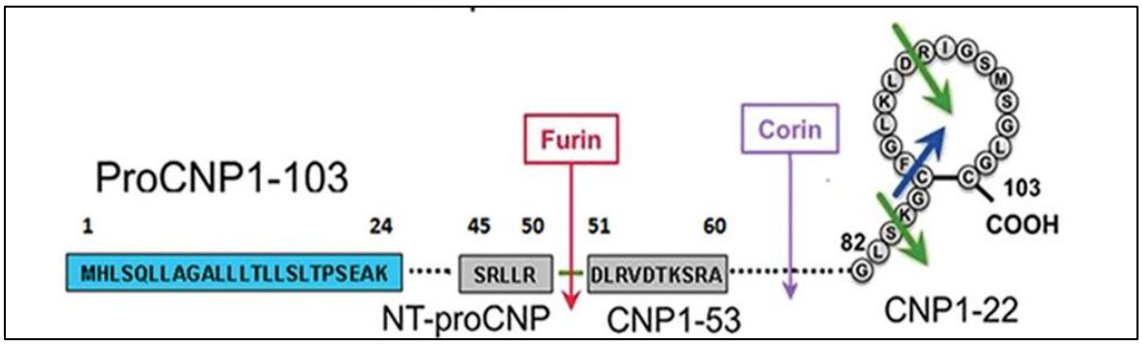

Figure (2.4): Schematic representation of the cleavage of pro-CNP to CNP (Volpe Rubattu, & Burnett, 2014).

The biological effects of the natriuretic peptides are summarized in Table (2.5) and Figure (2.5) (Nishikimi, Maeda, & Matsuoka, 2006).

Table (2.5): Biological effect of the natriuretic peptides (Nishikimi et al. 2006; Zhou & Wu, 2013).

1. Renal Action
Glomerulus
Dilatation of afferent arteriole and constriction of efferent arteriole
Relaxation of mesangial cells
Renal Tubules
Diuresis
Natriuresis
2. Vasodilation
3. Hormone
Inhibition of renin secretion
Inhibition of aldosterone synthesis
4. Cell Growth, Proliferation
Inhibition of proliferation in vascular smooth muscle cells, mesangial cells, cardiac fibroblasts,
Inhibition of hypertrophy in cardiac myocytes
5. Bone
Endochondral ossification
6. Central Nervous System
Inhibition of drinking
Inhibition of salt
Hypotensive action
Inhibition of vasopressin secretion
Inhibition of ACTH secretion

ACTH, adrenocorticotropic hormone

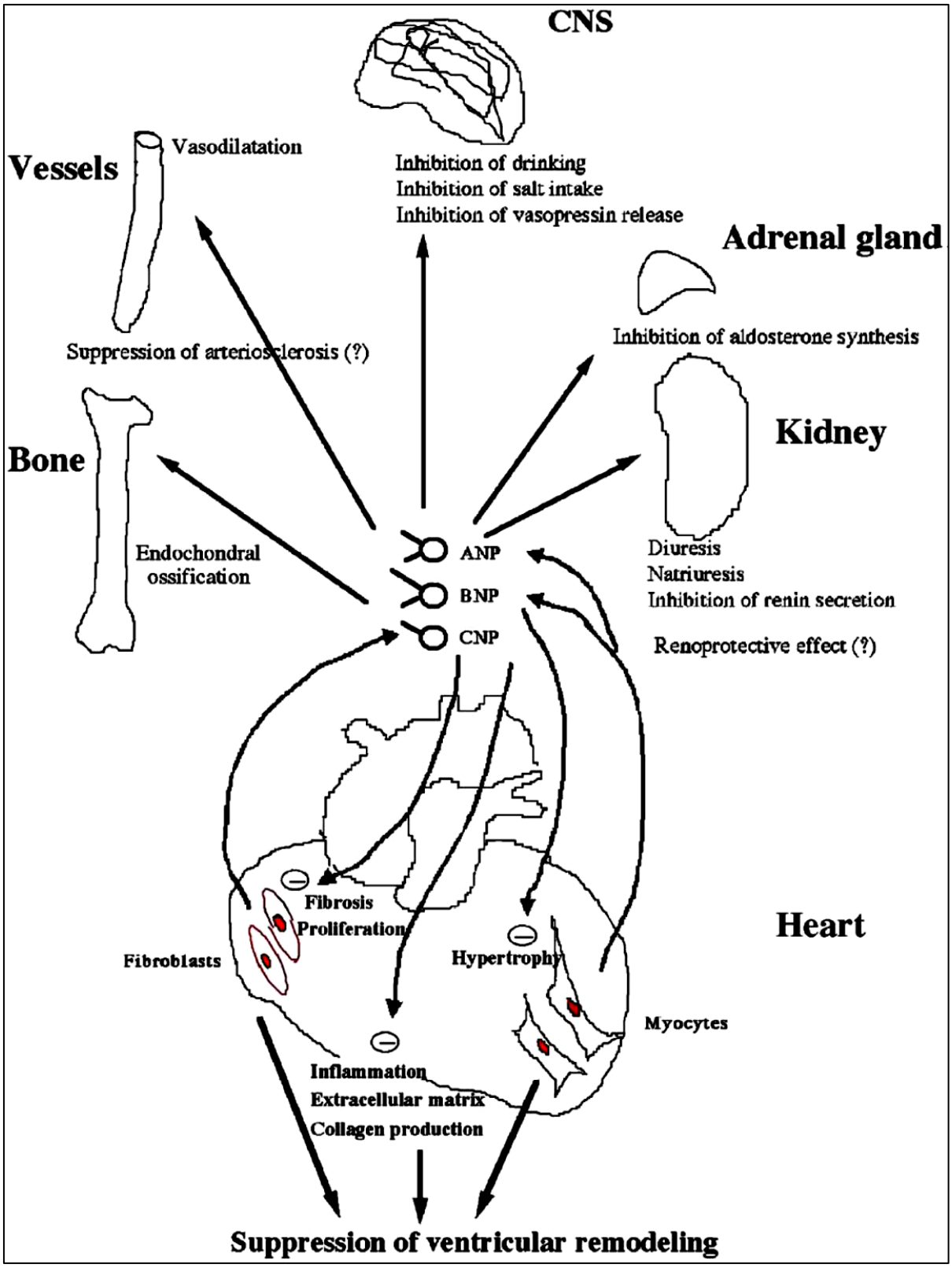

Figure (2.5): Schematic representation of the action and mechanism of natriuretic peptides (ANP, BNP, and CNP) in cardioprotection (Nishikimi et al., 2006).

2.3.2 Natriuretic Peptide Receptors

Natriuretic peptide receptor-A (NPR-A) and natriuretic peptide receptor-B (NPR-B) are guanylyl cyclase-linked, and they utilize cyclic guanosine monophosphate (cGMP) as the intracellular messenger. Both ANP and BNP bind preferentially to NPR-A, whereas CNP preferentially binds to NPR-B. All three natriuretic peptides bind to the third receptor, known as natriuretic peptide receptor-C (NPR-C). NPR-C is not linked to guanylyl cyclase (GC-A), but shows to act to clear the natriuretic peptides from the circulation (Staffel, Valletta, Federlein, Ehm, & Volkmann, 2016).

Thus, the natriuretic peptide system consists of three ligands and three receptors. These peptides cause effects such as diuresis, natriuresis, vasodilation, and inhibition of aldosterone synthesis and renin secretion as a circulating hormone, and thereby play an important role in regulating blood pressure and blood volume. The intensity of actions varies through the three peptides. ANP and BNP are each produced within the heart and secreted in response to stretching of muscles that typifies an increase in blood volume. The release of ANP and BNP from the heart has the most quickest biologic effect of increasing electrolyte and water excretion in the kidney by functionally antagonizing the "salt-sparing" role of the renin–angiotensin–aldosterone system. However, ANP and BNP also regulate the permeability of the systemic vasculature, cellular growth, cellular proliferation, and, as shown more recently, cardiac hypertrophy. Accumulating evidence suggests that the three natriuretic peptides act not only as circulating hormones but also as autocrine and/or paracrine factors. (Nishikimi et al., 2006).

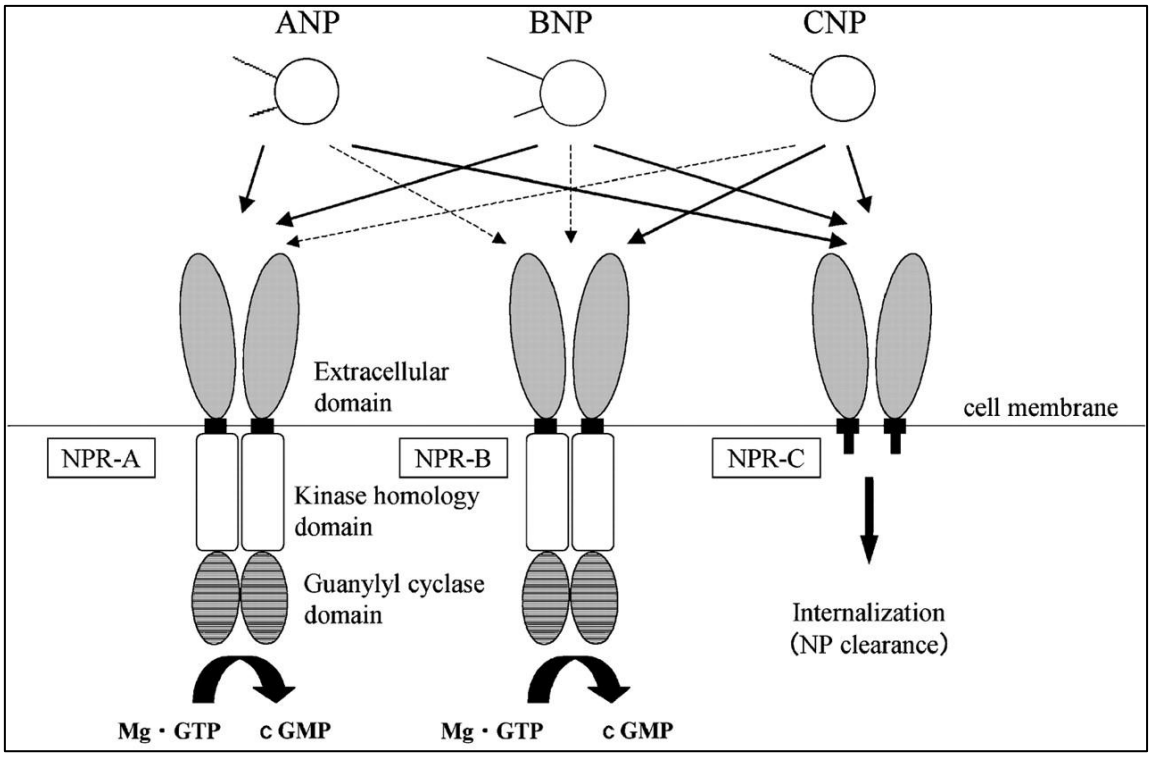

Figure (2.6): Schematic illustration of the interaction of three natriuretic peptides and three natriuretic peptide receptors. ANP: atrial natriuretic peptide, BNP: brain B-type natriuretic peptide, CNP: C-type natriuretic peptide, NPR-A: natriuertic receptor-A, NPR-B: natriuertic receptor-B, NPR-C: natriuertic receptor-C (Nishikimi et al., 2006).

2.4 Corin

2.4.1 Biology, Structure and Functional Role of Corin

Corin, a serine protease, is generally regarded as the main activator of natriuretic peptides, via cleavage from propeptide to active form of both ANP and BNP (Semenov, Tamm, Seferian, Postnikov, & Karpova, 2010). Corin is synthesized as a zymogen, which in turn demands activation by cleavage of a conserved site. The corin activator has not been identified. It is highly expressed in cardiomyocytes, and its promoter shares many of the same transcription binding sites as ANP and BNP precursors. Thus, it could be predicted that corin and natriuretic peptides would be up regulated in response to identical stimuli (Dong, Chen, Wang, Zhou, & Wu Q., 2012).

Corin is a type II transmembrane serine protease of 1042 amino acids that processes natriuretic peptides in the heart (Yan, Sheng, Seto, Morser, & Wu Q., 1999). It consists of an N-terminal cytoplasmic tail, a transmembrane domain, and an extracellular part

with a C-terminal trypsin-like protease domain. The transmembrane domain anchors corin on the surface of cardiomyocytes (Qi, Jiang, Zhu, & Wu, 2011). Corin has been demonstrated to be the "pro–A-type natriuretic peptide/pro–B-type natriuretic peptide convertase" that uniquely processes the natriuretic peptide precursor molecules into biologically active molecules (Yan, Wu F, Morser, & Wu Q., 2000). The enzyme is expressed primarily in atrial and ventricular cardiomyocytes, where it converts inactive pro-ANP and pro-BNP to active peptides. These peptides mediate their biological actions after binding to the natriuretic peptide receptor A and generating the second messenger cGMP to promote natriuresis, diuresis, and vasodilation (Figure 2.7) (Wu et al., 2009).

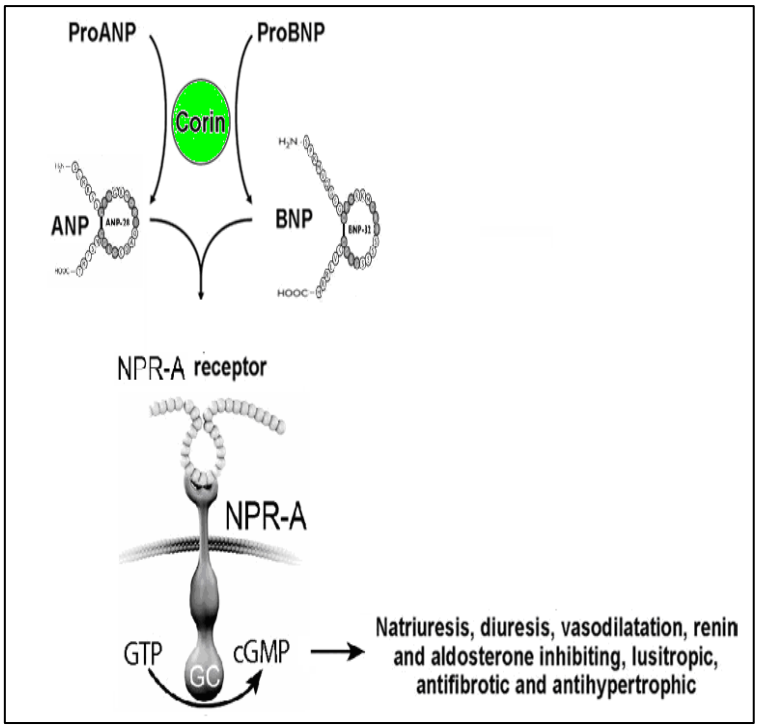

Figure (2.7): Schematic illustration of the processing of the two pro-cardiac natriuretic peptides by corin to biologically active mature cardiac natriuretic peptides (Burnett & Olson, 2007).

The physiological significance behind corin shedding is unclear; however, this process may involve a regulatory mechanism aimed at avoiding exaggerated proteolytic activity of corin **(**Armaly, et al 2013**)**.

2.4.2 Renal Corin Expression

Staining procedures were performed on rat kidney sections to analyze the intrarenal distribution of corin. Positive corin immunostaining was found in epithelial cells, with segmental expression in the proximal tubule, TAL, connecting tubule, and throughout the CD. Highest expression level was observed in the medulla. In the proximal tubule, corin staining was found in the apical endocytic compartment. In TAL (identified by NKCC2 staining), corin was expressed in vesicles around the nucleus and within the cytoplasm. Similarly, in CD (identified by aquaporin-2 (AQP2) staining) corin was localized evenly distributed in cytoplasmic vesicles. Interstitial cells were also positive for corin (Polzin et al., 2010).

2.4.3 Corin and Chronic Kidney Diseases

In the kidney, corin expression was located in the proximal tubule, TAL, connecting tubule and collecting duct, Also renal corin expression was significantly decreased in rat models of kidney disease, which may participate to sodium retention in those animals (Creemers et al., 2007 and Polzin et al., 2010 & Ichiki et al., 2011). In additional, corin may have a regional function in the kidney in regulating sodium excretion (Fang et al., 2013). Consistent with this, impaired sodium excretion and salt-sensitive hypertension were found in corin knockout mice (Wang et al., 2012).

Several studies, both in vitro and animal models, have demonstrated that corin deficiency was responsible for sodium excretion impairment (Ricciardi, Lacquaniti, Cernaro, Bruzzese, & Visconti, 2016).

2.4.4 Influence of Corin in the Nephrotic Kidney.

1. Signaling effects downstream of corin in the normal kidney. Corin, produced in the kidney, will cleave pro-ANP to make active ANP. ANP will bind to its receptor, natriuretic peptide receptor A (NPR-A), activating a guanylyl cyclase (GC-A) that will cleave guanosine triphosphate (GTP) and produce cGMP. cGMP (1) promotes afferent vasodilation and efferent vasoconstriction to increase glomerular filtration; (2) promotes retrieval of (AQP2) from the apical membrane; (3) depresses epithelial sodium channel (ENaC) activity; (4) activates cGMP dependent protein kinase II (PKGII), which

depresses renin release; and (5) binds to and activates phosphodiesterase 5 (PDE5), which feeds back to inhibit the cGMP. The overall effect is natriuresis and diuresis.

2. Signaling effects downstream of corin in the nephrotic kidney. Corin is reduced, resulting in decreased cleavage of pro-ANP to ANP. The decreased ANP levels result in decreased cGMP production. The trickle-down effect results in decreased GFR, increased AQP2, increased ENaC, increased PKG (explanation unknown), and increased PDE5 and phospho-PDE5 (pPDE5) (an apparently paradoxical response, since the lower cGMP would be further lowered by the action of the excessive PDE5). (Klein, 2010).

In animal model, over expression of corin has been linked to renal anti-fibrotic effects, through an increased intracellular cGMP synthesis, inhibiting collagen synthesis and proliferation of fibroblasts. To date, the association between corin and renal disease progression was not in depth estimated (Ricciardi et al., 2016).

Previously, Polzin et al., 2010 reported that the renal expression and function of corin. They found by Immunohistochemical analysis that corin localized with ANP and the nephrotic and glomerulonephritic models exhibited concomitantly increased pro-ANP and decreased ANP protein levels in the kidney consistent with low amounts of corin. They were concluded that the kidneys from corin knockout mice had increased amounts of renal β-ENaC and its activators, phosphodiesterase (PDE) 5 and protein kinase G II, when compared to wild-type mice. A similar expression profile was also found in cell culture suggesting the increase in PDE5 and kinase G II could account for the increase in β-ENaC seen in nephrotic syndrome and GN. Thus, they suggested that corin might be involved in the salt retention seen in glomerular diseases. Also Klein, (2010) was suggested corin (an ANP protease) that may regulate sodium reabsorption in nephrotic syndrome.

CKD patients had markedly reduced urinary corin levels and this reduction correlated with disease riskiness. By immunostaining, human corin protein was identified on the epithelial cell surface in renal tubules. The renal corin mRNA and protein levels were significantly lower in CKD patients than non-CKD controls. These results point that renal tubular corin may be shed into urine and that urinary and renal

corin levels were reduced in CKD patients. They suggested that reduced corin levels in the kidney may explain the underlying pathology in CKD (Fang et al., 2013).

2.4.5 Previous Studies:

1- The study of reduced urinary corin levels in patients with CKD (Fang et al., 2013); confirmed that human corin protein was identified on the epithelial cell surface in renal tubules by immunostaining, also the renal corin mRNA and protein levels were significantly lower in CKD patients than non-CKD controls.

2- Corin a new player in the regulation of salt–water balance and blood pressure, by Armaly, et al (2013); is another study assured that natriuretic peptides are a serious endocrine system in the regulation of body fluid balance and blood pressure, and corin mediates an important step in the cascade of natriuretic peptide biosynthesis.

3- The findings from the study of: Predicting progression in CKD; corin balances heart and renal systems by Ricciardi et al., (2016) ; are clearly demonstrated that high levels of corin were associated with accelerated progression of kidney disease. Corin emerged as an independent predictor of renal endpoints, providing prognostic information in addition to well-established risk markers, such as proteinuria, uric acid or markers of cardiac dysfunction.

Chapter 3

Subject and Methods

Chapter 3
Subject and Methods

3.1 Study Design

The present study is a case-control investigation. Case-control studies are often used to identify factors that may contribute to a medical condition by comparing subjects who have that condition/disease (the "cases") with subjects who do not have the condition/disease but are otherwise similar (the "controls"). Case-control studies are quick, widely used, relatively inexpensive to implement, require comparatively fewer subjects, and allow for multiple exposures or risk factors to be assessed for one outcome (Song & Chung, 2010).

3.2 Target Population

The study population was comprised patients with CKD male children aged less than 12 years attending kidney unit at Abdel Aziz al-Rantisi Hospital Gaza strip. Control group was an equal number of an age matched and in residence place.

3.3 Sample Size

CKD male children were aged less than 12 years taken from kidney unit at Abdel Aziz al-Rantisi hospital in Gaza Strip. Control healthy individuals were selected from the general population. Cases and controls were matched for age. The sample size calculations were based on the formula for case-control studies, by using EPI-INFO statistical package version 3.5.1 (EPI-INFO, 2008) was used with 95% CI, 80% power and 50% proportion as conservative and OR > 2. (Epidemiological Program Office (Epi Info, Version 3.5.1). (2008): Atlanta, Georgia (USA), WHO-CDC. The sample size in case of 1:1 ratio of case control was found to be 43:43.

3.4 Inclusion Criteria

All of CKD male children, regardless treatment protocol were included.

3.5 Exclusion Criteria

- Cases and controls whose aged above 12 years old.
- Subjects with a history of cancer.
- Patients with other chronic diseases such as diabetes.
- Patients who take hormone replacement therapy or corticosteroid therapy.
- Patient with liver cirrhosis, heart disease, hematologic disorder or malignant disease were excluded from the study to eliminate potential confounding factors which may influence heart function and plasma biomarkers.

3.6 Ethical Consideration

The necessary approval to conduct the study was obtained from Helsinki Committee in the Gaza Strip. Coordination with the Ministry of Health (MOH) was fulfilled. Parents of the participants were given a full explanation about the purpose of the study, assurance about the confidentiality of the information obtained through the questionnaire and blood analysis, and that they have the right to refuse to participate or to drop out in any phase of the study.

3.7 Data Collection

3.7.1 Questionnaire Interview

A meeting interview was used for filling in a questionnaire which designated for matching the study need. All interviews were conducted face to face by the researcher herself. During the study, the interviewer explained to the Parents of the participants any of the confused questions that may not clear to them. Most questions were yes/no question which offers dichotomous choices and multiple choice (Backestrom & Hursh-Cesar, 2012). The questionnaire included questions on sociodemographic data (Age, education, employment, family income/month,family history of kidney disease, and clinical data (Age at diagnosis and duration of kidney disease).

3.7.2 Body Mass Index

Body mass index (BMI) was calculated as the ratio of body weight in Kg/height in meter square. The subjects were asked to remove shoes and heavy clothes before measurement of weight and height.

3.7.3 Specimen Collection and Biochemical Analysis

Blood samples were collected from CKD male children and controls. Venous blood sample (about 5 ml) was drawn by the researcher herself into vacutainer tubes from each control and CKD individuals. The blood was left for a while without anticoagulant to allow blood to clot, the serum samples were obtained by centrifugation at room temperature at 4000 rpm/10 minutes for biochemical analysis**.** Serum Creatinine, Urea, Uric acid, total protein, albumin, phosphorus, calcium, sodium, potassium, triglycerides, cholesterol and corin were analyzed.

3.8 Calculated Measurements

- **Glomerular filtration rate** was calculated by Schwartz equation:

  GFR (ml/min/1.73m^2) = 0.55x length / serum creatinine (Muhaisen, Sharif, & Yassin, 2012)

- **Calculation of chemical tests** for urea, creatinine, uric acid, cholesterol triglycerides, total protein, albumin, phosphorus were performed by the auto analyzer automatically according to beer's law after calibration and adjustment of the photometers against water blank using a specific program of every test inserted to the instrument.

$$\text{The concentration of colorimetric test} = \frac{\text{Abs. of test x concentration of standard}}{\text{Abs. of standard}}$$

3.9 Materials and Instruments

3.9.1 Chemicals and Reagents

Chemicals and reagents used in this study are shown in the following Table:

Reagent	Supplier
Urea	Lab kit, India
Creatinine	Coral Clinical Systems, India
Uric Acid	Spectrum, Egypt
Total Protein	Coral Clinical Systems, India
Albumin	Cromatest, Spain
Cholesterol	Globe Diagnostics, Italy
Triglycerides	Spectrum, Egypt
Phosphorus	Lab kit, India
Corin	RnDSystems, USA

3.9.2 Instruments

The main equipments that were used are:

ERBA300
Nova 10
Centrifuge
Deep Freezer -20C
Water Bath
Vortex Mixer
Micropipettes
ELISA reader
Shaker

3.10 Biochemical Analysis

3.10.1 Determination of Serum urea

Serum urea was cleaved enzymatically into NH_4^+ and CO_2. NH_4^+ reacted with α-ketoglutarate in a reaction catalyzed by GLDH with simultaneous NADH to NAD^+ (Kaplan A., 1984).

Principle

$$\text{Urea} + H_2O + 2H^+ \xrightarrow{\text{Ureasee}} 2(NH_4^+) + CO_2$$

$$\alpha\text{-ketoglutarate} + NH_4^+ + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-Glutamate} + NAD^+ + H_2O$$

The decrease in NADPH absorbance is proportional to urea level in the sample.

Table (3.1): Reagents used in determination of serum urea**.**

Reagent	Concentration
A : TRIS PH (7.8) α - ketoglutarate Urease	80 mmol/l 6 mmol/l 75000 u/l
B: NADH GLDH	0.32 mmol/l 60000 u/l
Standard	50 mg/dl

Assay Procedure

The working solution was prepared by mixing 4 parts of A with 1 part of B to obtain working reagent.

Analytical Procedure:

For urea estimation at 340 nm wave length was used with 1cm optical path at 37 ºC incubation the measurement were taken against blank as described below.

Reaction: Fix time (decrease).

Monoreagent Procedure:

	Blank	Standard	Sample
Working reagent	1000µl	1000µl	1000µl
Standard	--	10 µl	--
Sample	--	--	10 µl

Mix, incubate 30 seconds at 37 °C, then reading A1 of sample, standard, blank, after precisely 60 seconds read absorbance A2

Determine:

ΔA= ((A1-A2)sample or standard)-((A1-A2)BLANK)

Calculation

ΔA = (A1 –A2) sample or standard:

$$\text{Urea (mg/dl)} = \frac{\Delta A \text{ sample X concentration of standard}}{\Delta A \text{ standard}}$$

Reference Value

Child	10 - 45 mg/dl

3.10.2 Determination of Serum Creatinine

Serum creatinine was determined by using Jaffa's kinetics method using coral reagent kits (Bowers, L.D., 1980).

Principle:

Picric acid in alkaline medium was reacted with creatinine to compose orange color complex with the alkaline picrate. The intensity of color formed during the fixed time is directly proportional to the amount of creatinine present in the sample.

Creatinine + alkaline Picrate → orange colored complex.

Reagents

Reagent
L1: picric acid reagent
L2: buffer reagent
S: Creatinine Standard (2mg/dl)

Working Reagent

The working solution was prepared by mixing equal volumes picric acid reagent and buffer reagent, the working reagent is stable at R.T.(25-30°C) for at least one week.

Procedure:

Wavelength: 520 nm.

Light path: 1cm

Temperature: 37 ºC

Addition sequence	Blank	Standard	Test
L1 reagent L2 reagent	0.5ml 0.5ml	0.5ml 0.5ml	0.5ml 0.5ml
Standard		100µl	
Sample			100µl

Mix well and read initial absorbance A1 for standard and test after exactly 30 seconds. Read another absorbance A2 of standard and test exactly after 60 seconds later. Calculate the change in absorbance ΔA for both the standard and test.

For standard ΔAS =A2-A1

For Test ΔAT =A2-A1

Calculation.

$$\text{Creatinine (mg/dl)} = \frac{\Delta A \text{ sample X concentration of standard}}{\Delta A \text{ standard}}$$

Reference value

Child	0.2 - 0.7 mg/dl

3.10.3 Determination of Serum Uric Acid:

Serum uric acid was determined by using uricase-POD enzymatic colorimetric endpoint method with 4-amino-antipyrine (Barham D. & Trinder P., 1972).

Principle:

The assay is based on the modified Trinder peroxidase assay utilizing 3,5-dichloro-2-hydroxybenzenesulfonic acid (DCHB). Uric acid is oxidized to allantoin by uricase with production of hydrogen peroxide. The peroxide reacts with 4-amino-antipyrine and DCHB in the presence of peroxidase to yield a quinoneimine dye. The subchange in absorbance at 546 nm (500-550nm) is proportional to uric acid concentration in the sample.

$$\text{Uric acid} + O_2 + H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$H_2O_2 + \text{4-AAP} + \text{DCHB} \xrightarrow{\text{POD}} \text{Quinoneimine} + H_2O$$

Reagents

Reagent	**Concentration**
Phosphate Buffer DCHB Potassium hexacyanoferrate 4-amino-antipyrine Peroxidase Uricase	100 mmol/l 5.0 mmol/l 80 µmol/l 0.6 mmol/l > 3000u/l > 500u/l
Standard	0.357 mmol/l (6 mg/dl)

Reagent Preparation:

Uric acid liquizyme single reagent is supplied ready to use.

Procedure:

Wavelength: 520 nm (500-550 nm)

Optical path: 1cm

Temperature: 30 ºC /37 ºC

Addition sequence	**Blank**	**Standard**	**Test**
Reagent	1.0 ml	1.0 ml	1.0 ml
Standard		20µl	
Sample			20µl

Mix and incubate for 5 minutes at 37 °C, or 10 minutes at 15-25 °C, read the absorbance of standard and test against blank within 30 minutes.

Calculation:

$$\text{Uric Acid (mg/dl)} = \frac{\text{Abs. sample X concentration of standard}}{\text{Abs standard}}$$

Reference Value

Child	2.0 – 5.5 mg/dl

3.10.4 Determination of Serum Total Protein:

Serum uric acid was determined by using Biuret colorimetric endpoint method (Gornall, A.G., 1949).

Principle:

Proteins, in an alkaline medium, bind with the cupric ions present in the biuret reagent to form a blue-violet colored complex. The intensity of the color formed is directly proportional to the amount of proteins in the sample.

Proteins + Cu^{++} ⟶ Blue Violet Colored Complex.

Reagents

Reagent	Concentration
Standard	8 g/dl
Biuret Reagent	

Reagent Preparation:

Biuret reagent is ready to use.

Procedure:

Wavelength: 550 nm

Optical path: 1cm

Temperature: 37 ºC

Addition sequence	Blank	Standard	Test
Reagent	1.0 ml	1.0 ml	1.0 ml
Standard		20µl	
Sample			20µl

Mix and incubate for 10 minutes at 37 °C, or 30 minutes at 15-25 °C, read the absorbance of standard and test against blank within 60 minutes.

Calculation.

Total protein (g/dl) = $\frac{\text{Abs. sample X concentration of standard}}{\text{Abs standard}}$

Reference Value

Child	6.0 – 8.0 g/dl

3.10.5 Determination of Serum Albumin:

Serum albumin was determined by using bromocresol green colorimetric endpoint method (Doumas, B. T., Watson, W. A. & Biggs, H. G. 1971).

Principle:

This method is based on the specific bindingof bromocresol green (BCG), an anionic dye and the protein at acid pH with the resulting shift in the absorption wavelength of the complex. The intensity of the color formed is proportional to the concentration of albumin in the sample.

BCG+ Albumin $\xrightarrow{\text{pH 4.3}}$ BCG-albumin Complex.

Reagents

Reagent	Concentration
Standard	5 g/dl
Bromocresol Reagent. succinate buffer 75 mmol/l, pH 4.2, BCG 0.12 mmol/l	

Reagent Preparation:

Bromocresol reagent is ready to use.

Procedure:

Wavelength: 630 nm

Optical path: 1cm

Temperature: 37 ºC

Addition sequence	Blank	Standard	Test
Reagent	2.0 ml	2.0 ml	2.0 ml
Standard		10µl	
Sample			10µl

Mix and incubate for 10 minutes at room temperature, read absorbance of standard and test against blank within 30 minutes.

Calculation.

$$\text{Albumin (g/dl)} = \frac{\text{Abs. sample X concentration of standard}}{\text{Abs standard}}$$

Reference Value

Child	3.8 – 5.4 g/dl

3.10.6 Determination of Serum Cholesterol:

Enzymatic colorimetric method was used for the quantitative determination of total cholesterol in serum, using Globe Diagnostics kit (Jakobs DS., et al., 1990).

Principle

The measurement is based on the following enzymatic reaction

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{4- amminoantipyridine} + \text{hydroxybenzoatered} \xrightarrow{\text{POD}} \text{complex} + 4\ H_2O$$

The intensity of the red complex is proportional to the total cholesterol in the sample.

Reagents Composition of Cholesterol Kit

Reagent	Concentration
Good's buffer (pH 6.7)	100 mmol/l
4- Amminophenazone	0.5 mmol/l
Cholesterol esterase (CHE)	> 300 U/l
Cholesterol oxidase (CHOD)	> 100U/l
Peroxidase (POD)	>200 KU/l
Hydroxybenzoic cid	20 mmol/l
NaN^3	≤0.095g/l
Standard	200 mg/dl

Reagent Preparation:

Cholesterol reagent is ready to use.

Procedure:

Wavelength: 510 nm

Optical path: 1cm

Temperature: 37 ºC

.

	Blank	Standard	Sample
Reagent	1.0 ml	1.0 ml	1.0 ml
Standard		10 µl	
Sample			10 µl

Mix and incubate for 10 minutes at 37ºC. Read the absorbance of standard and samples at 510 nm against blank. Colour is stable for 60 minute, protected from light.

Calculation:

$$\text{Cholesterol (mg/dl)} = \frac{\text{Abs. sample X concentration of standard}}{\text{Abs standard}}$$

Reference values for cholesterol concentration in serum

Recommended value	< 200 mg/dl
Upper limits	200-239 mg/dl
High values	>240 mg/dl

3.10.7 Determination of Serum Triglycerides:

GPO-PAP enzymatic colorimetric method was used for the quantitative determination of triglycerides in serum, using Spectrum kit (Bucolo G., David H., 1973).

Principle of Serum Triglycerides Test:

The method is based on the hemolysis of serum triglycerides to glycerol and free fatty acid by lipoprotein lipase (LPL). The glycerol is then phosphorylated by adenosin triplphosphate in the presence of Glycerolkinase (GK) to compose glycerol -3-phosphate (G-3-P) and adenosine diaphosphate (ADP). G-3-P is oxidized by glycerolphosphate oxidase (GPO) to form dihydroxyacetone phosphate (DHAP) and hydrogen peroxide (H_2O_2). Ared chromgen is produced by peroxidase (POD) catalyze coupling of 4–aminoantipyrine (4-AAP) and phenol with hydrogen peroxide (H_2O_2), proportional to concentration of triglyceride in sample

$$\text{Triglycerides} \xrightarrow{\text{LPL}} \text{Glycerol + Fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-phosphate + ADP.}$$

$$\text{Glycerol-3-phosphate + } O_2\text{Dihydroxyacetone} \xrightarrow{\text{GPO}} \text{phosphate + } H_2O_2.$$

$$2H_2O_2 + \text{4-chlorophenol + 4-AAP} \xrightarrow{\text{POD}} \text{Quinoneimine dye + } 4H_2O.$$

Reagents Composition of Triglycerides Kit

.

Reagent	Concentration
PipesBuffer (pH 7.0)	50 mmol/l
ATP	1.0 mmol/l
4-chlorophenol	6.0 mmol/l
Glycerol kinase (GK)	> 750 U/l
Peroxidase(POD)	> 2.0 KU/l
lipase	> 10 KU/l
4-Aminoantipyrine	1.0 mmol/l
Glycerol-3-phosphate-oxidase (GPO)	> 3.5 KU/l
Magnesium aspartate	>0.5 mmol/l
Sodium Azide	8.0 mmol/l
Standard	200 mg/dl

Procedure:

Wavelength: 546 nm (500 - 550 nm)

Optical path: 1cm

Temperature: 37 ºC

	Blank	standard	Sample
Reagent	1.0 ml	1.0 ml	1.0 ml
Standard		10 µl	
Sample			10 µl

Mix and incubate for 5 minutes at 37°C. Read the absorbance of standard and samples at 546 nm against blank. Color is stable for 30 minute, protected from light.

Calculation:

$$\text{Triglycerides (mg/dl)} = \frac{\text{Abs. sample X concentration of standard}}{\text{Abs. standard}}$$

Reference Values for Serum Triglycerides

Triglyceride	Risk classification
< 150 mg/dl	Normal
150-199 mg/dl	Borderline high
200-499 mg/dl	High

3.10.8 Determination of Serum Phosphate:

Inorganic phosphate was determined by using Phosphomolybdate U.V. method (Farrell E. C., et al 1984).

Principle:

Inorganic phosphate reacts in acid medium with ammonium molybdate to form a phosphomolybdate complex with yellow color. The intensity of the color formed is directly proportional to the amount of inorganic phosphate in the sample.

Proteins + Cu^{++} ⟶ Blue Violet Colored Complex.

Reagents

Reagent	Concentration
Standard	5 g/dl
Molybdic Reagent	
Ammonium molybdate	0.4 mM
Sulphuric acid	210 mM
Detergents	

Reagent Preparation:

Molybdic reagent is ready to use.

Procedure:

Wavelength: 340 nm

Optical path: 1cm

Temperature: 37 ºC

Addition sequence	Blank	Standard	Test
Reagent	1.0 ml	1.0 ml	1.0 ml
Standard		10µl	
Sample			10µl

Mix and incubate for 5 minutes at 37 °C, read absorbance of standard and test against blank within 30 minutes.

Calculation.

$$\text{Phosphorus (mg/dl)} = \frac{\text{Abs. sample X concentration of standard}}{\text{Abs. standard}}$$

Reference value

Child	4.0 – 7.0 mg/dl

3.10.9 Determination of Serum Corin.

Serum corin was determined by quantitative enzyme linked immunoassay (ELISA).

Principle of ELISA Test.

The enzyme immunoassay is also called sandwich assay. It utilizes specific and high affinity monoclonal antibody for human corin in samples bind to the immobilized antibody on micro titer plate. Standards and samples are pipptted into the wells and any corin present is bound by the immobilized antibody. After washing, any enzyme-linked polyclonal antibody specific for human corin is added to the wells. Following a wash, a substrate solution is added and color develops quantitatively depend on the corin level of the sample. The color development is stopped and the intensity of the color is measured.

Table (3.2): Composition of reagents for ELISA corin kit

Human Corin Microplate	96 well polystyrene microplate 12 strips coated with a monoclonal antibody specific for human corin
Human Corin Standard	Recombinant human corin in a buffered protein solution with preservatives lyophilized contain recombinant corin standard and have to be reconstituted with RD6-1 dilution buffer
Human Corin Conjugate	12.5 ml of polyclonal antibody specific for human corin conjugated to horseradish peroxidase with preservatives
Assay Diluent RD1-41	12 ml of a buffered protein base with preservatives
Calibrator Diluent RD6-1	21 ml of buffered animal serum with preservatives
Wash Buffer Concentrate	21 ml of a 25-fold concentrated solution of buffered surfactant with preservatives
Color Reagent A	12 ml of stabilized hydrogen peroxide
Color Reagent B	12 ml of stabilized chromogen (tetramethylbenzidine)
Stop Solution	23 ml of diluted hydrochloric acid
Plate Sealers	4 adhesive strips

Preparation of Reagents for ELISA Test:

Before running the test, prepare the following:

Wash Buffer:

If crystals have formed in the concentrate, we have to warm to room temperature and mix gently. Add 20 ml of wash buffer concentrate to deionized or distilled water to prepare 500 ml of wash buffer.

Substrate Solution:

Color reagent A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Human Corin Standard – Refer to the Vial Label for Reconstitution Volume:

Reconstitute the human corin standard with calibrator diluents RD6-1. This reconstitution produces a stock solution of 4800 pg/ml. Then we mix well the standard and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Then we pipette 200 µL of calibrator diluent RD6-1 into each tube. We used the stock solution to produce a dilution series. The 4800 pg/ml standard serves as the high standard and the calibrator diluent serves as the zero standard.

Sample Preparation

Samples require a 2-fold dilution. A suggested 2-fold dilution is 75µL of sample + 75µL of calibrator diluents RD6-1.

Analytical Procedure for ELISA Test

Assay Procedure

All reagents and samples were being obtained to room temperature. For optimal results, accurate pipetting and adherence to the protocol are recommended.

1- All reagents, working standards, and samples were prepared as directed in the previous sections.

2- Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

3- 50µl of Assay Dilution RD1-41was added to each well.

4- 50µl of Standard and samples were added per well. The adhesive strip was covered and incubated for 2 hours at room temperature on a horizontal orbital microplate shaker set at 500 rpm.

5- After incubation; we aspirated each well and wash 3 times by filling each well with 400µl Washing Buffer. Complete removal of liquid at each step is essential to good performance. Following the last washing step, we removed any remaining wash buffer by aspirating or decanting. The plate was inverted and blotted it against the clean paper.

6- 100µl of Human Corin Conjugate was added to each well. We covered the wells with a new adhesive stripe, and incubate the plate for 2 hours at room temperature on the shake.

7- After incubation the wells, we repeated the aspiration/wash as step 5.

8- Following the last washing step 100 µl of Substrate Solution was added to each well and incubates for 30 minutes at room temperature on the bench top, protected from light.

9- The reaction was stopped by adding 100 µl Stopping Solution to all wells. The color in the wells should change from blue to yellow. Gently we taped the plate to ensure thorough mixing.

10- The absorbance was measured within 30 minutes at 450 nm.

Calculation

- Plot the standard concentrations on the x-axis versus the mean value of the absorbance of the standards on the y-axis.
- Using the mean absorbance value for each sample to determine the corresponding concentration from the standard curve.

Table (3.3): Sample values of corin

Sample Type	Mean [pg/ml]	Standard Deviation(SD) [pg/ml]	Range [pg/ml]
Serum n=43	1359	442.2	480 - 2200

3.11 Statistical Analysis

Data were computer analyzed using SPSS/ PC (Statistical Package for the Social Science Inc. Chicago, Illinois USA, statistical package version 22.0)

- Simple distribution of the study variables and the cross tabulation were applied.
- T test was applied.
- Chi-square (χ^2) was used to identify the significance of the relations, associations, and interactions among various variables.
- Pearson's correlation test was applied.

- The results in all the above mentioned procedures were accepted as statistically significant when the p-value was less than 5% ($p \leq 0.05$).
- Range as minimum, maximum values, the mean and standard deviation were used.
- Graph by Excels.
- The basic ANOVA "F test" was used.
- The percentage difference was calculated according to the formula: Percentage difference equals the absolute value of the change in value, divided by the average of the 2 numbers, all multiplied by 100.

  Percent difference = | (V1 - V2) | / ((V1 + V2)/2)) X 100

Chapter 4

Results

Chapter 4

Results

4.1 General Characteristics of Study Population

Table 4.1 summarizes general characteristics of study population. The study included 43 cases and 43 controls. Age was matched between cases and controls, so age was classified into 4 groups, less than 3 years, 3 to less than 6, 6 to less than 9 and from 9 to 12 years. The number of subjects was 9 (20.9%), 12 (27.9%). 10 (23.3%) and 12 (27.9%) for each group in cases and controls, respectively. There was no significant difference between cases (χ^2=0.000, P=1.000). The distribution of cases according to the stages of CKD were 10 (23.3%) stage 1, 3 (7.0%) stage 2, 13 (30.2%) stage 3, 12 (27.9%) stage 4 and 5 (11.6%) stage 5.

Table (4.1): General characteristics of study population

General characteristics	Controls (n=43) n (%)	Cases (n=43) n (%)	χ^2	P-value
Age (years)				
(<3)	9 (20.9)	9 (20.9)	0.000	1.000
(3 to <6)	12 (27.9)	12 (27.9)		
(6 to <9)	10 (23.3)	10 (23.3)		
(9-12)	12 (27.9)	12 (27.9)		
CKD stage				
Stage 1		10 (23.3)		
Stage 2		3 (7.0)		
Stage 3		13 (30.2)		
Stage 4		12 (27.9)		
Stage 5		5 (11.6)		

*P-value significant at P≤0.05; χ^2 : chi-square test; CKD: Chronic Kidney Diseased

4.2 Baseline Characteristics, Onset CKD and Systolic and Diastolic Blood Pressure among Study Population

Table 4.2 illustrates age, weight, height, BMI, onset CKD and systolic and diastolic blood pressure among the study population. The mean of cases age was 6.1±3.6 years compared to 6.6±3.3 years of controls. The statistical test was shown no significant difference in the mean age between cases and controls (-7.9 % difference, t=

-0.616 and P=0.540). Similarly, there was no significant difference in the mean height between cases and controls (103.2±26.4 versus 110.6±19.6 cm, -6.9% difference, t= -1.476 and P=0.144). On the other hand, there was a statistically significant difference in weight between cases and controls (18.8±11.0 versus 26.0±10.1 kg, -32.1 % difference, t = -3.143 and P=0.002). Therefore, the difference in BMI between cases and controls was highly significant (16.1±2.7 versus 20.6±3.5 kg/m^2, -24.5% difference, t = -6.581 and P<0.001). The systolic blood pressure (SBP) was significantly higher in cases than controls (103.9±13 versus 95.7±0.8.2 mmHg, 8.2% differences, t=3.480 and P=0.001). However the diastolic blood pressure (DBP) was significantly lower in cases than controls (61.7±13.3 versus 66.3±5.8 mmHg, -7.2 % differences, t= -2.092 and P=0.040).

Table (4.2): Baseline Characteristics, onset CKD and systolic and diastolic blood pressure among study population

Parameters	**Controls (n=43)** Mean±SD	**Cases (n=43)** Mean±SD	**% difference**	**t**	**P-value**
Age (years) Range (min-max)	6.6±3.3 (0.5-12)	6.1±3.6 (0.5-12)	-7.9	-0.616	0.540
Onset CKD (years) Range (min-max)		8.0±2.8 (0.5-12)			
Height (cm) Range (min-max)	110.6±19.6 (76-150)	103.2±26.4 (49-162)	-6.9	-1.476	0.144
Weight (kg) Range (min-max)	26±10.1 (9.0-48.0)	18.8±11 (2.8-54.7)	-32.1	-3.143	0.002*
BMI (kg/m^2) Range (min-max)	20.6±3.5 (13.3-25.8)	16.1±2.7 (11-24.5)	-24.5	-6.581	<0.001*
SBP(mmHg) Range (min-max)	95.7±8.2 (77-110)	103.9±13 (74-124)	8.2	3.480	0.001*
DBP(mmHg) Range (min-max)	66.3±5.8 (55-78)	61.7±13.3 (33-90)	-7.2	-2.092	0.040*

*P-value significant at P≤0.05; **CKD**: chronic kidney diseases; **SPB**: systolic blood pressure; **DBP**: diastolic blood pressure and **BMI**: body mass index.

4.3 Kidney Function Test among Study Population

The Kidney function tests among the study population are summarized in table 4.3. The means of serum urea, creatinine and uric acid were significantly higher in cases compared to controls (80.7±46.8 versus 23.9±5.7 and 108.6%, P<0.001, 1.9±1.5 versus 0.4±0.1 and 130.4%, P<0.001, 5.5±2.6 versus 4.1±1.0 and 29.2%, P=0.001 respectively). On the other hand, the mean of GFR was found to be significantly higher in controls compared to cases (143.2±31.9versus 57.8±51.4, % of differences= -85.0, t= -9.252 and P<0.001).

Table (4.3): Kidney function tests among study population

Kidney function	**Controls (n=43)** Mean±SD	**Cases (n=43)** Mean±SD	**% difference**	**t**	**P-value**
Urea (mg/dl) Range (min-max)	23.9±5.7 (10-42)	80.7±46.8 (9-196)	108.6	7.895	<0.001*
Creatinine (mg/dl) Range (min-max)	0.4±0.1 (0.2-0.7)	1.9±1.5 (0.2-6.3)	130.4	6.293	<0.001*
Uric acid (mg/dl) Range (min-max)	4.1±1 (2.4-6.8)	5.5±2.6 (1.5-13.4)	29.2	3.292	0.001*
GFR (ml/min/1.73m²) Range (min-max)	143.2±31.9 (77.4-211.9)	57.8±51.4 (7.2-206.3)	-85.0	-9.252	<0.001*

*P-value significant at P≤0.05 and **GFR**: Glomerular filtration rate

4.4 Total Protein, Albumin, Cholesterol and Triglyceride among Study Population

Table 4.4 demonstrated serum corin, total protein, albumin, cholesterol and triglyceride among the study population. Serum corin was progressively increased showing mean of 1816.3±78.3 in cases and mean of 1359.1±442.2 in controls. The SPSS test showed a significant difference in the means level of corin among cases and controls (t=3.336 and P=0.001). The differences in total protein mean levels among controls and cases was statically significant (t= -3.716, P<0.001). On the other hand, the differences in the mean of albumin between cases and controls were not significant (P=0.351). The mean of cholesterol was found to be significantly higher in cases

compared to controls (195.4±80.4 versus159.7±28.9 mg/dl, % of differences=20.1, t=2.744 and P=0.007). In addition, triglyceride was significantly higher in cases than in controls (173.1±133.1versus108.4±42.9, % difference=-46.0, t=3.031 and P=0.003).

Table (4.4): Corin, Total protein, albumin, Cholesterol and Triglyceride among study population

Parameters	**Controls (n=43)** Mean±SD	**Cases (n=43)** Mean±SD	**% difference**	**t**	**P-value**
Corin (Pg/ml) Range (min-max)	1359.1±442.2 (480-2200)	1816.3±782.3 (1000-4910)	28.8	3.336	0.001*
Total protein (g/dl) Range (min-max)	7.2±0.6 (6.1-8.3)	6.4±1.3 (3-8.2)	-11.8	-3.716	<0.001*
Albumin (g/dl) Range (min-max)	4.3±0.4 (3.6-5.2)	4.1±1.1 (1.2-6)	-4.8	-0.938	0.351
Cholesterol (mg/dl) Range (min-max)	159.7±28.9 (119-247)	195.4±80.4 (83-448)	20.1	2.744	0.007*
Triglyceride (mg/dl) Range (min-max)	108.4±42.9 (46-222)	173.1±133.1 (48-870)	46.0	3.031	0.003

*P-value significant at P≤0.05.

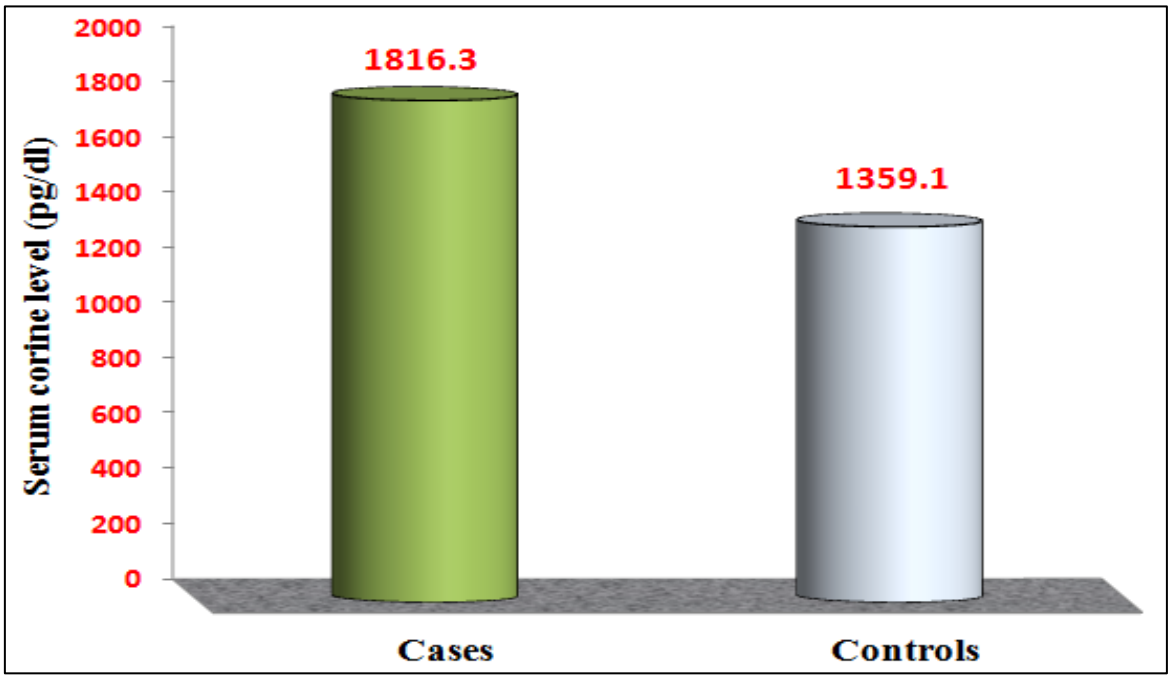

Figure (4.1): Distribution the mean of serum corin level (Pg/ml) among controls and cases.

4.5 Electrolytes among Study Population

Table 4.5 points out that the mean serum phosphorus was not significantly between cases and controls (5.5±1.3 versus 5.1±0.6, % difference=7.5, t=1.696, P=0.094). Similarly trend was found for the potassium level (4.2±0.9 versus 4.3±0.3, % difference= -2.4, t= -0.840, P=0.403).In addition, there was no significant difference in the chloride level between cases and controls (111.1±7.2 versus 108.9±2.5, %difference=2.0, t=1.914 and P=0.059). However, the sodium mean was lower in patients than controls (140.7±4.7 versus 142±2.6, %difference= -1.5). There was a statically significant difference between patient and control groups (t = -2.647, P =0.010). Similarly there was a significant difference in the calcium level between cases and controls (9.1±1.2 versus 10.6±0.9, %difference= -15.2, t= -6.626 and P<=0.001).

Table (4.5): Electrolytes among study population

Electrolytes	**Controls (n=43)** Mean±SD	**Cases (n=43)** Mean±SD	**% difference**	**t**	**P-value**
Ph (mmol/L)	5.1±0.6	5.5±1.3	7.5	1.696	0.094
Range (min-max)	(3.8-6)	(2.4-9.3)			
Na (mmol/L)	142.8±2.6	140.7±4.7	-1.5	-2.647	0.010*
Range (min-max)	(139-148)	(126-149)			
K (mmol/L)	4.3±0.3	4.2±0.9	-2.4	-0.840	0.403
Range (min-max)	(3.9-5)	(2.4-6.1)			
Ca (mg/dL)	10.6±0.9	9.1±1.2	-15.2	-6.626	<0.001*
Range (min-max)	(9-12.7)	(5.9-12.4)			
CL (mmol/L)	108.9±2.5	111.1±7.2	2.0	1.914	0.059
Range (min-max)	(103-115)	(92-124)			

*P-value significant at P≤0.05; **Ph**: phosphorus; **Na**: sodium; **K:** potassium; **Ca**: calcium and **CL**: Chloride.

4.6 Distribution of Serum Corin Level According to General Characteristics of Study Population

Table 4.6 compares serum corin level according to general characteristics of study population. The mean levels corin was found to be non-significant according to age (P=0.382), and CKD stages (P=0.086).

Table (4.6): Distribution of Serum corin level according to general characteristics of study population

General characteristics	Serum corin level (Pg/ml) Mean±SD (min-max)	F	P-value
Age (years)			
>3	1817.8±821.3 (680-4200)	1.043	0.382
(3 to <6)	1551.4±534.6 (480-2260)		
(6 to <9)	1580.0±371.5 (1000-2480)		
(9-12)	1455.8±843.1 (600-4910)		
CKD stage			
Stage 1	1490.0±444.0 (1000-2260)	2.211	0.086
Stage 2	2730±1918.3 (1300-4910)		
Stage 3	1850.4±832 (1020-4200)		
Stage 4	1644.6±380.1 (1100-2200)		
Stage 5	2244±675 (1750-3330)		

*P-value significant at P≤0.05, CKD: Chronic kidney disease.

4.7 Correlation between Serum Corin Level and Baseline Characteristics, Onset CKD and Systolic and Diastolic Blood Pressure among Study Population

Table 4.7 gives the relationship of serum corin and baseline characteristics, onset CKD, systolic and diastolic blood pressure among the study population.

Serum corin exhibited a significant negative correlations with age (r= -0.216 and P=0.046).Similarly corin showed negative significant correlations with height, weight, and BMI (r=-0.321, P=0.003; r=-0.305, P=0.004, and r=-0.247, P=0.022, respectively). However, the negative correlation between corin and diastolic blood pressure was not significant (r=-0.161, P=0.140) The Pearson correlation test showed positive

correlations with onset CKD and systolic blood pressure but not significant (r=0.174, P=0.264 and r=0.029, P=0.789, respectively).

Table (4.7): Correlation between serum corin level and Age, weight, height, BMI, onset CKD, SBP and DBP among study population

Parameters	**Serum corin level (Pg/ml)**	
	Pearson correlation (r)	**P-value**
Age (years)	-0.216	0.046*
Onset CKD (years)	0.174	0.264
Height (cm)	-0.321	0.003*
Weight (kg)	-0.305	0.004*
BMI (kg/m^2)	-0.247	0.022*
SBP(mmHg)	0.029	0.789
DBP(mmHg)	-0.161	0.140

*P-value significant at P≤0.05; **CKD**: chronic kidney diseases; **SPB**: systolic blood pressure; **DBP**: diastolic blood pressure; **BMI**: body mass index.

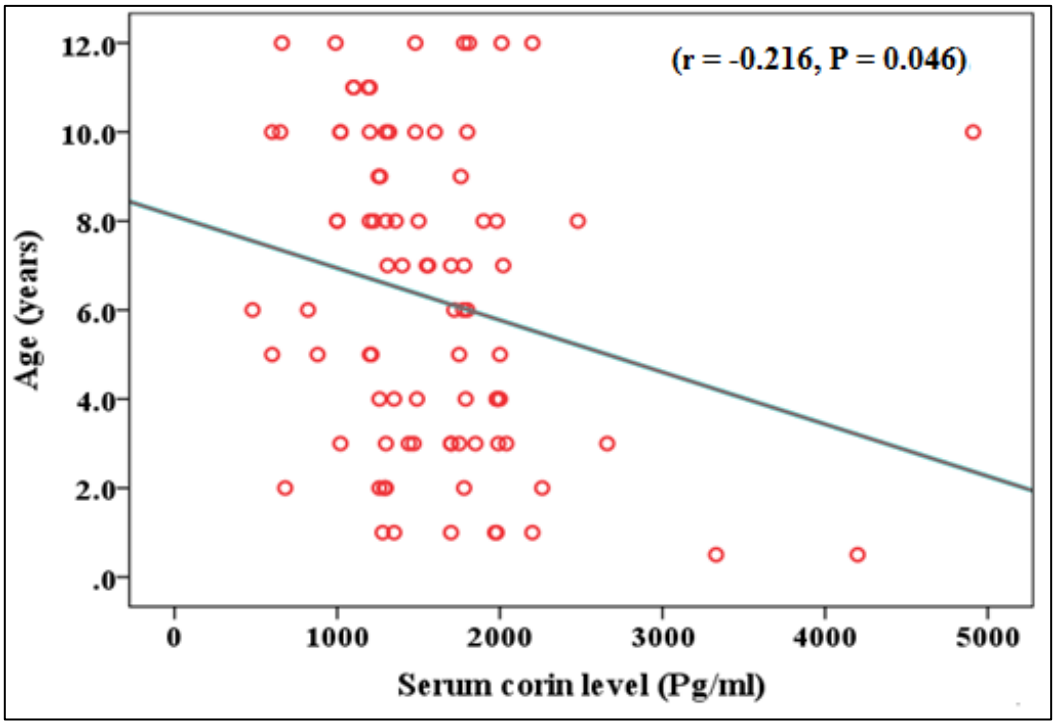

Figure (4.2): Negative significant correlation between serum corin and age among the study population.

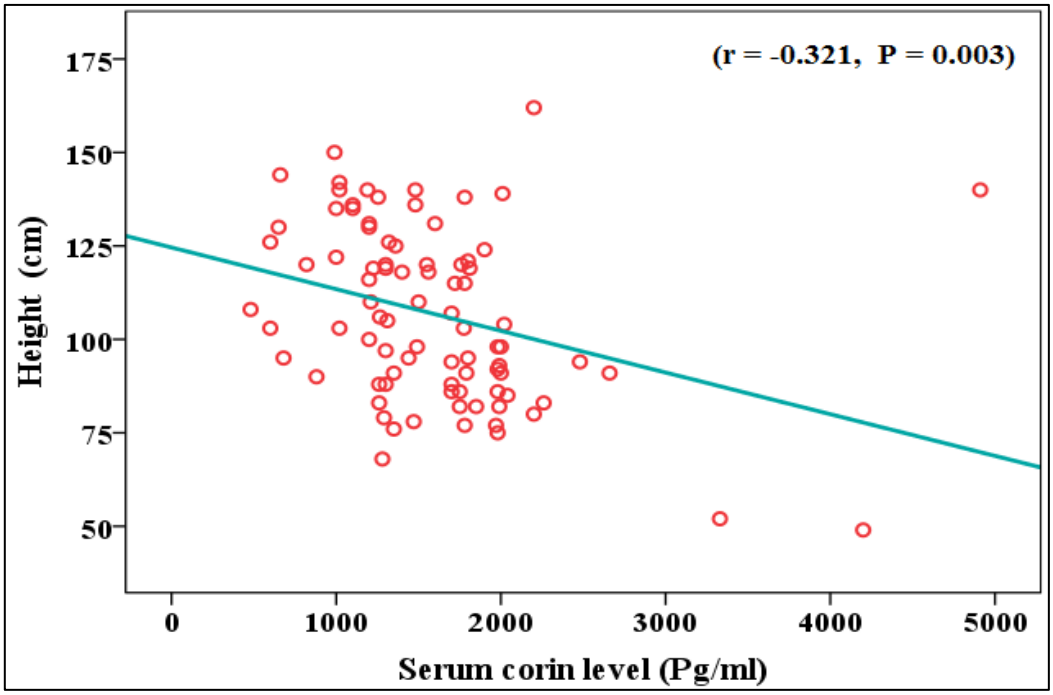

Figure (4.3): Negative significant correlation between serum corin and height among the study population.

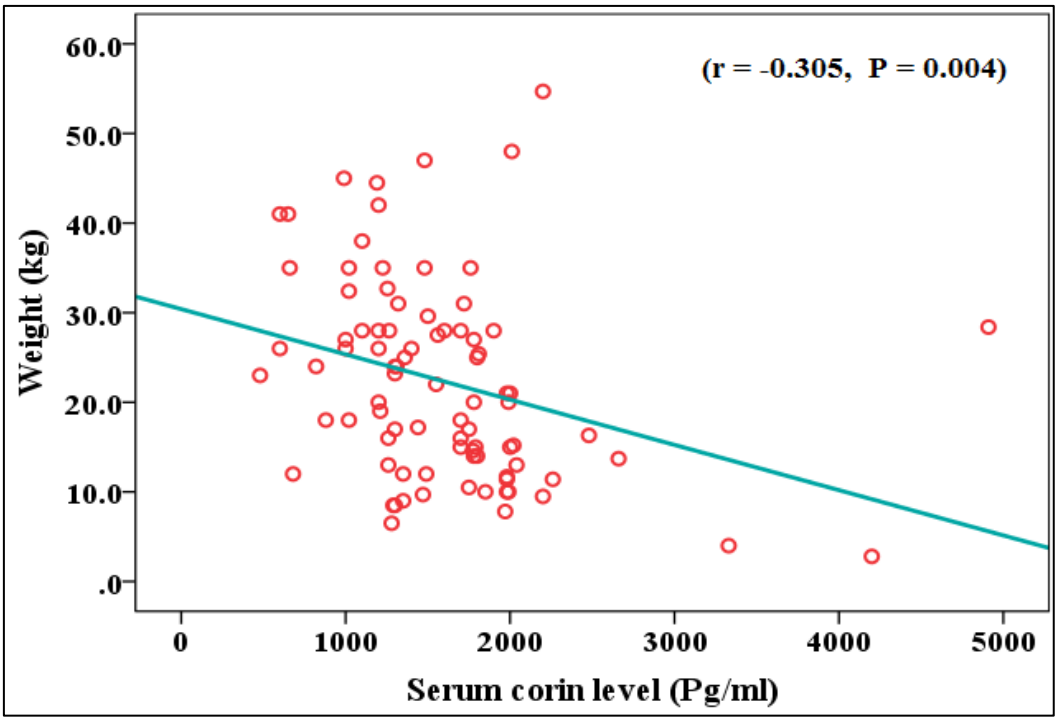

Figure (4.4): Negative significant correlation between serum corin and weight among the study population.

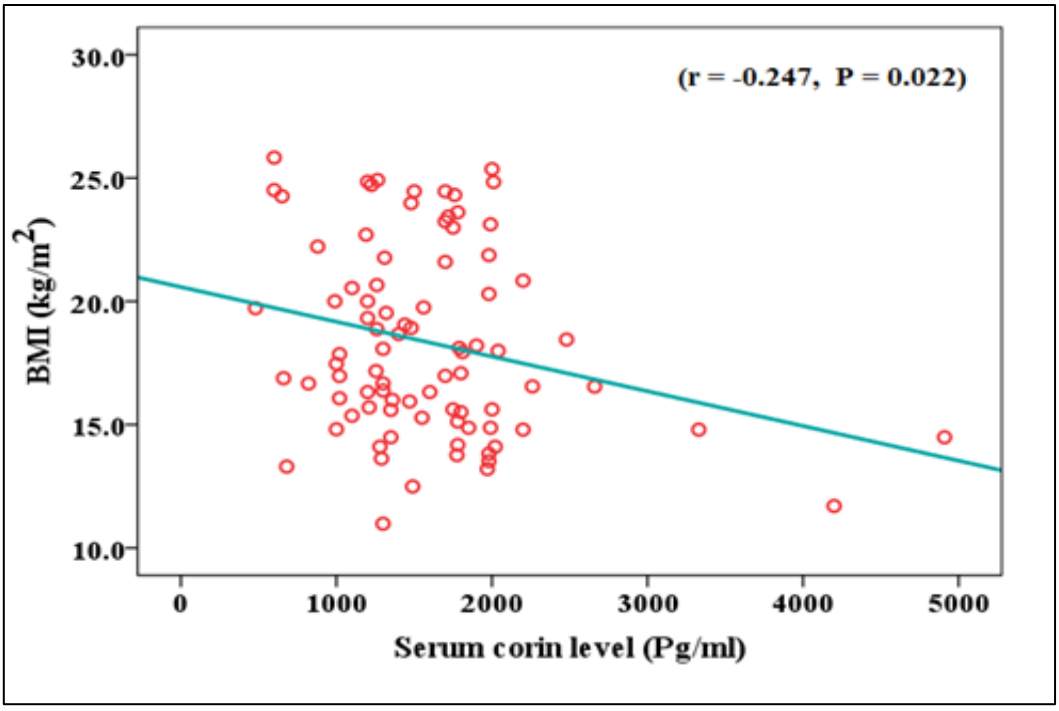

Figure (4.5): Negative significant correlation between serum corin and BMI among the study population.

4.8 Correlation between Serum Corin Level and Kidney Function Test among Study Population

The relation between serum corin level and kidney function test among the study population is pointed out in table 4.8. The Pearson correlation test showed positive significant correlation between corin level and urea (r=0.224, P=0.038). However, no significant correlation was found between corin and creatinine(r= 0.182 and P=0.094). Similarly, corin showed positive with no significant correlations with uric acid(r=0.100, P=0.361). There was an increased significant negative correlation between corin and GFR (r=-0.360, P=0.001, figure 4.7).

Table (4.8): Correlation between serum corin level and kidney function test among study population

Kidney Function test	**Serum corin level (Pg/ml)**	
	Pearson correlation (r)	**P-value**
Urea (mg/dl)	0.224	0.038*
Creatinine (mg/dl)	0.182	0.094
Uric acid (mg/dl)	0.100	0.361
GFR (ml/min/1.73m²)	-0.360	0.001*

*P-value significant at P≤0.05; **GFR**: Glomerular filtration rate.

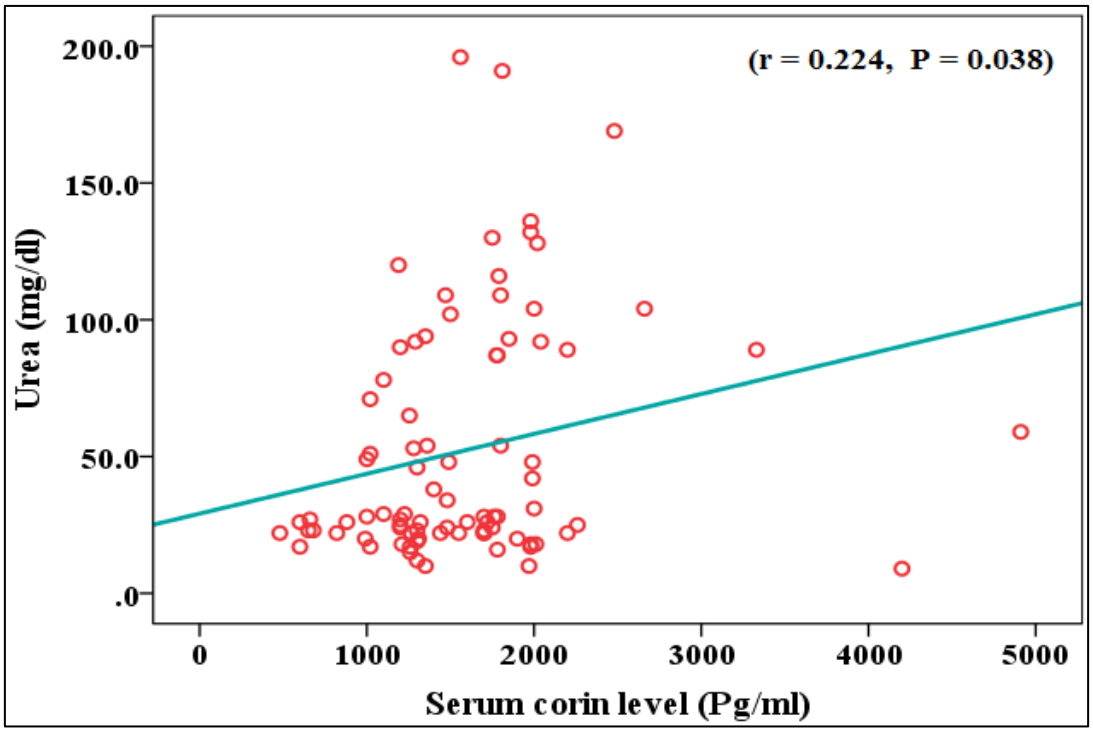

Figure (4.6): Positive significant correlation between serum corin and urea among the study population.

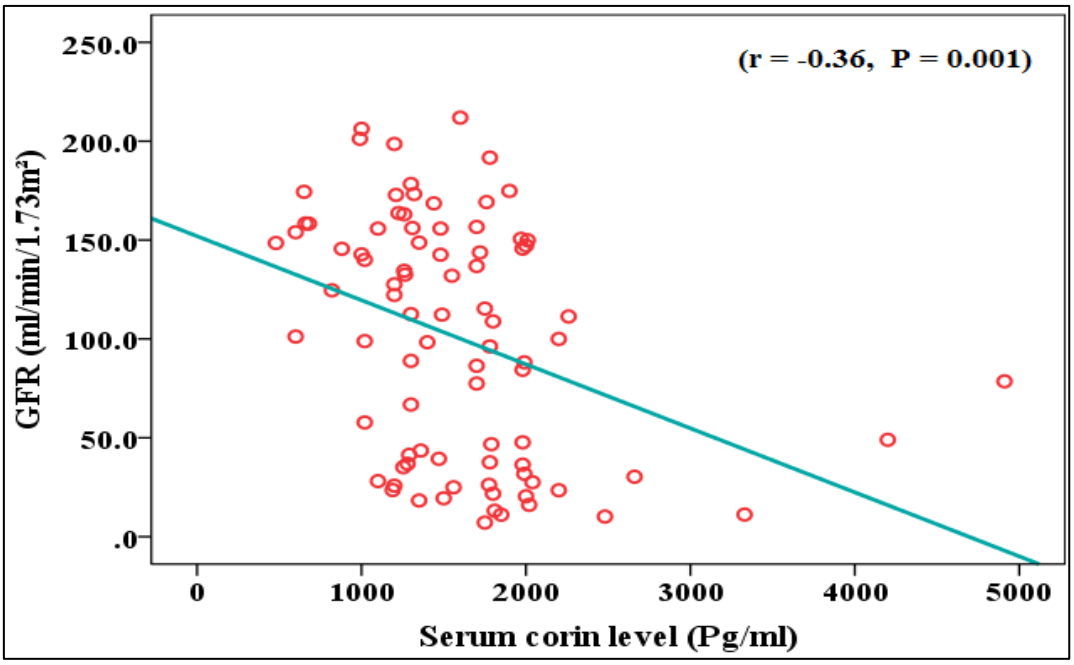

Figure (4.7): Negative significant correlation between serum corin and GFR among the study population.

4.9 Correlation between Serum Corin Level and Total Protein, Albumin, Cholesterol and Triglyceride among Study Population

Table 4.9 illustrates the results of Pearson correlation between serum corin level and total protein, albumin, cholesterol and triglyceride among the study population. Negative significant correlation was found for corin with total protein (r=-0.306 and P=0.004). On the other hand, corin level showed negative correlations but not statically significant with albumin (r = -0.188, P=0.083). However, corin level showed positive correlations but not statically significant with cholesterol and triglyceride (r = -0.137, P=0.209 and r=0.093 and P=0.393, respectively).

Table (4.9): Correlation between serum corin level and total protein, albumin, cholesterol and triglyceride among study population

Parameters	Serum corin level (Pg/ml)	
	Pearson correlation (r)	P-value
Total protein (g/dl)	-0.306	0.004*
Albumin (g/dl)	-0.188	0.083
Cholesterol (mg/dl)	0.137	0.209
Triglyceride (mg/dl)	0.093	0.393

*P-value significant at P≤0.05.

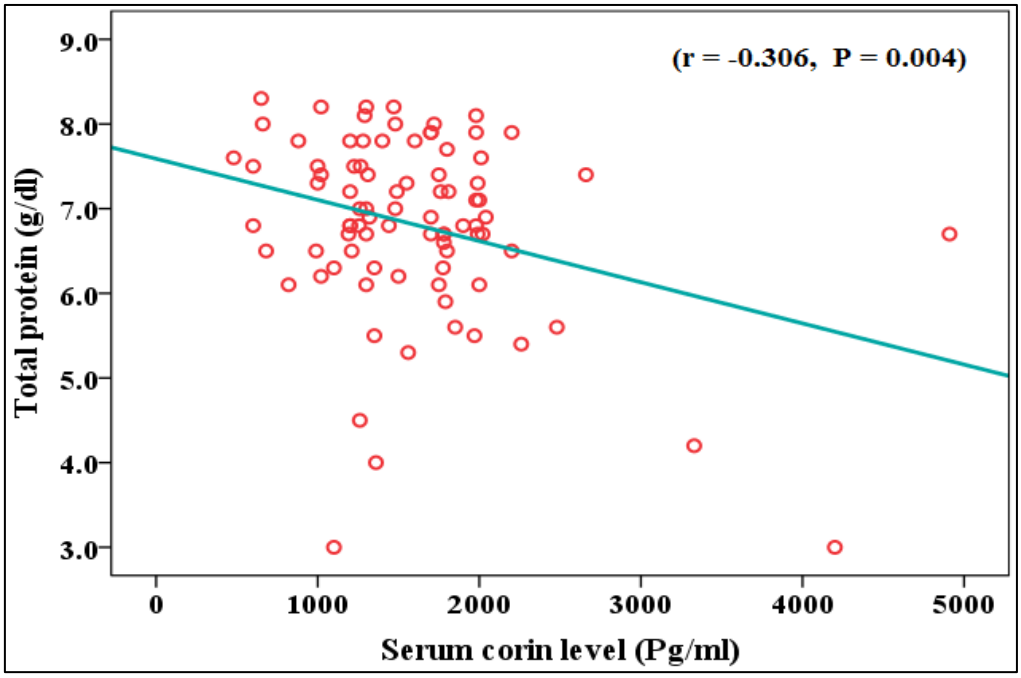

Figure (4.8): Negative significant correlation between serum corin and total protein among the study population.

4.10 Correlation between Serum Corin Level and Electrolytes Parameters among Study Population

Serum corin in relation to electrolytes parameters including phosphorus, sodium, potassium total calcium and chloride of the study population is summarized in table 4.10. Pearson correlation test revealed positive significant correlations between corin and phosphorus (r=0.254, P=0.018). On the other hand, there was a negative significant correlation between corin and calcium (r=-0.227, P=0.035).Sodium showed negative non-significant correlation with serum corin (r=-0.152and P=0.163). However, there was no significant correlation between potassium and chloride with serum corin (r=0.131 and P=0.231 and r=0.051 and P=0.641, respectively).

Table (4.10): Correlation between serum corin level and electrolytes parameters among study population

Electrolytes	Serum corin level (Pg/ml)	
	Pearson correlation (r)	**P-value**
Ph (mmol/L)	0.254	0.018*
Na (mmol/L)	-0.152	0.163
K (mmol/L)	0.131	0.231
Ca (mg/dL)	-0.227	0.035*
Cl (mmol/L)	0.051	0.641

*P-value significant at P≤0.05; **Ph**: phosphorus; **Na**: sodium; **K**: potassium; **Ca**: calcium and **CL**: Chloride.

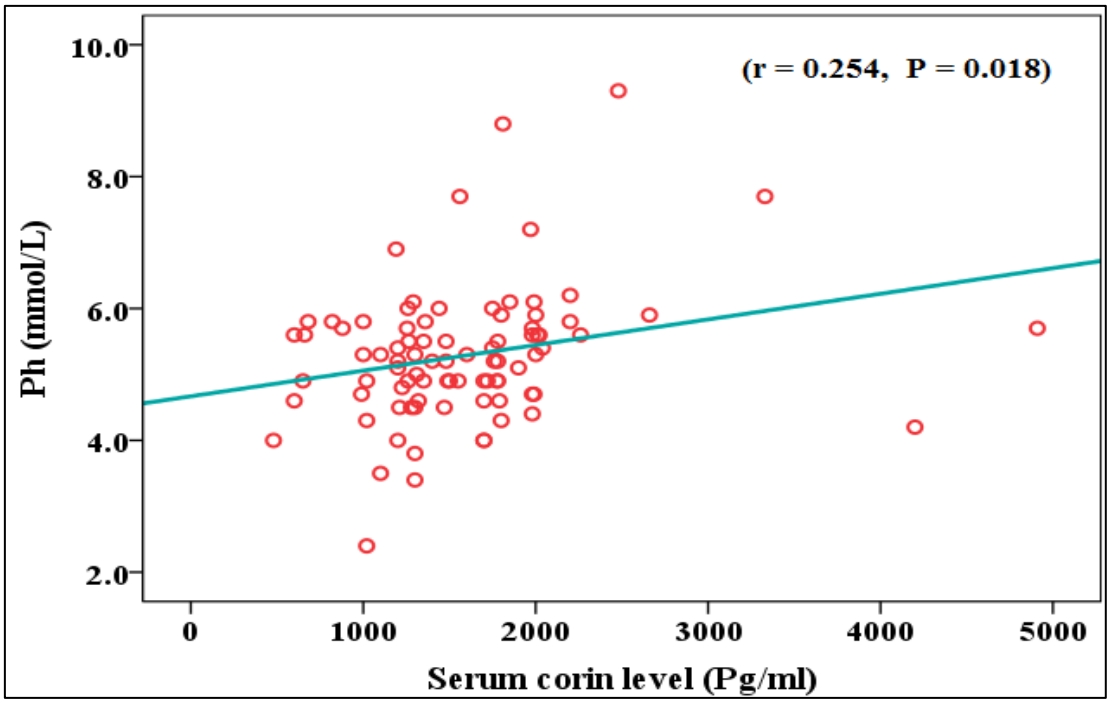

Figure (4.9): Negative significant correlation between serum corin and phosphorus among the study population.

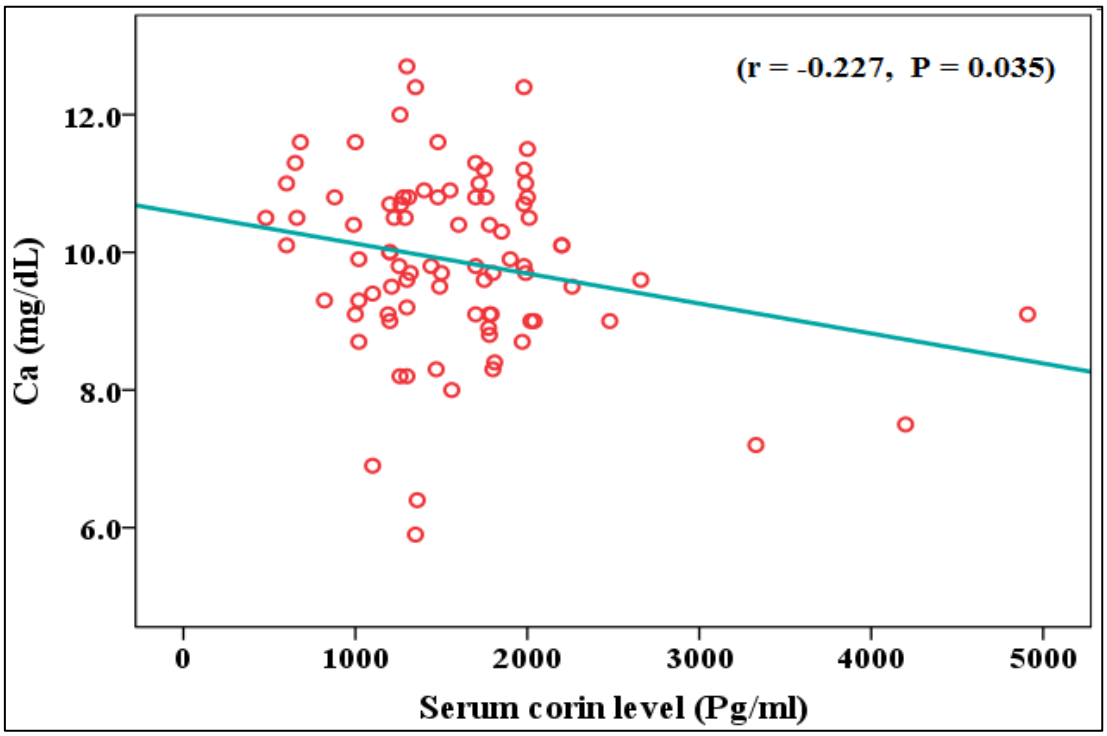

Figure (4.10): Negative significant correlation between serum corin and calcium among the study population.

Chapter 5

Discussion

Chapter 5

Discussion

CKD is a clinical syndrome characterized by a gradual loss of kidney function over time. The incidence of among CKD children is escalating worldwide and, consequently, this has become a major health care problem. Moreover, CKD is major effect factor on healthcare costs and economic of the family (Warady, Abraham, Schwartz, Wong, & Muñoz, 2015). Corin is a cardiac protease that regulates blood pressure by activating natriuretic peptides. Recent animal studies identified corin expression in the kidney where it may regulate renal function (Fang et. al., 2013).

In the Gaza Strip, data on CKD were limited to annual reports emerged from the Palestinian Ministry of Health. In addition, there are no data available on the development of the disease towards different stages of nephropathy. To our knowledge, this is the first study which investigated serum corin levels in CKD patients, revealing higher corin levels than in normal control. These data could be apparently that serum corin levels were found high in CKD patients that are not affected by heart failure or high blood pressure. This finding is not confirmed by previous studies.

This necessitates a further assessment of corin status and some biochemical parameters among CKD male children in Gaza strip. Although few studies were have been assessed some early markers of CKD. Therefore, the present study is the first to serum corin levels in different CKD stages. However, corin level in females was not investigated due to variations in normal values in which males have many times higher than females (Dong et al., 2012 and Peleg, Ghanim, Vered, & Hasin, 2013).

This study was done in Al-Rantisi pediatric hospital in Gaza Strip which considers the main pediatrics governmental hospital in Gaza Strip, in order to understand the relationship between serum corin levels and CKD and to provide reliable information that may help in investigating the association of serum corin levels with the development of CKD in children. The present study has some limitations that should be mentioned. It was a single-center and hypothesis-generating study, involving a relatively small cohort of patients. Moreover, we did not examine natriuretic peptide levels, which can reflect the direct activity of corin. Also, we could not examine the corin levels in urine samples.

5.1 Personal Profile among the Study Groups:

The study populations were matched in gender and age because previous studies on serum corin levels were known to be affected by gender and age (Ichiki et al., 2011and Peleg et al., 2013). As the studied groups matched for gender and age they were given reliable comparable results. The target population was 43 cases and 43 controls males below 12 years old, we adjust this factor to cases and control according to age groups

As indicated in the present study, there was significant correlation found between serum corin and age among the study population. Similar results were found by Ichiki et al., (2011), Peleg et al., (2013) and Fathy et al., (2015). However, there was no association found between serum corin and age groups (>3, 3 to <6, 6 to <9 & 9-12) among study population which agree with Dong, Chen, Yang, He, and Liu, (2010) and Shrestha, Troughton, Borowski, Yandle, & Richards, (2010) found no association between corin and age groups.

This study showed that the prevalence of CKD stage 3 was highest followed by Stages 4, then stage 1, 5 and 2 stage in Gaza strip, which agree with others reported (Muhaisen et al., 2012). In addition, no association between CKD stage and serum corin levels in our study. No previous studies were done about CKD stage and serum corin levels.

5.2 Baseline Characteristics, Onset CKD and Systolic and Diastolic Blood Pressure among Study Population

The present study demonstrated shown no association between height in cases and controls. In contrast, patients with CKD were lower weight and BMI compared to controls. This indicated decreasing in weight and BMI associated with CKD. This study was in line with that of Lu, Molnar, Naseer, Mikkelsen, & Kalantar-Zadeh, (2015) and Rodenbach, Schneider, Furth, Moxey-Mims, & Mitsnefes, (2015), they found a significant relation between decreases in BMI with CKD.

The mean of onset CKD was about 8 years in the study population because inclusion criteria were CKD male children aged less than 12 years. The systolic and diastolic blood pressure was significantly higher in cases than controls. Our study agrees with Rossignol, Massy, Azizi, Bakris, & Ritz, (2015) who reported CKD

associated with patient's left ventricular (LV) diastolic and diastolic dysfunction in CKD patients. As we noted both studied groups cases and controls have normal systolic and diastolic blood pressure because both groups considered children age and no had complication due to CKD, many studied illustrated CKD repeated complication will development in adult age (Sarnak, Bloom, Muntner, Rahman, & Saland,(2015) and Brück, Stel, Gambaro, Hallan, & Völzke, 2016).

5.3 Kidney Function Test among Study Population

As indicated in our data serum urea and creatinine concentrations of CKD were significantly higher compared to that of controls. Urea is formed by the liver as an end product of protein breakdown and is one marker of the kidney function (Higgins, 2016). The increase in serum urea observed here due to impairment in its filtration in kidney and may be as a result of impaired hepatic function and/or due to a disturbance in protein metabolism (Kirtane, Leder, Waikar, Chertow, & Ray, 2005 and Pietrement, Gorisse, Jaisson, & Gillery, 2013). Creatinine is a waste product that is normally filtered from the blood and excreted in the urine. Increase creatinine levels in CKD-related to disturbance of kidney function (Methven, Gasparini, Carrero, Caskey, & Evans, 2017). In addition, the observed increase in urea and creatinine explained on the basis of glomerular hyperfiltration (GFR) due to decrease creatinine clearing from blood (Hsu, Xie, Waikar, Bonventre, & Zhang, 2017 and Levey, Becker, & Inker, 2015). However, higher uric acid was associated with CKD; the uric acid is the end product of an exogenous pool of purines and endogenous purine metabolism, so increase in serum uric acid in CKD patient indicated to difficult in clarified it from blood due to damage in renal functions (Feig, 2014 and Jalal, Decker, Perrenoud, Nowak, & Bispham, 2017).

GFRs the best test to measure your level of kidney function and determine the stage of kidney disease (Schwartz, Muñoz, Schneider, Mak, & Kaskel, 2009). In the current study, lower GFR was associated with CKD compared to healthy subject. Our results agree with Böhm, Ezekowitz, Connolly, Eikelboom, & Hohnloser, (2015) and Dharmarajan Bragg-Gresham, and Morgenstern, Gillespie, & Li, (2017). They concluded decrease in GFR among CKD due to failure in ability kidney to filtration waste from blood CKD patients. Also, it is possible that, in CKD patients, inflammatory reactions may damage vessel walls and increase the glomerular permeability. In

addition the physiological actions of the natriuretic peptides in the kidney which increased GFR by inducing vasodilation of afferent arteriole and vasoconstriction of efferent arteriole. (Armaly et al., 2013).

5.4 Serum Corin among Study Population

Corin is a type-II transmembrane serine protease that is highly expressed in both endothelial and myocardial cells in the heart (Jiang, Wu, Wang, Chen, & Peng, 2011). Both corin and furin are involved in cleave pro-atrial natriuretic peptide (ANP) and pro-BNP into their active forms (ANP and BNP) (Semenov et al., 2010). Corin is potentially involved in hypertension and cardiac hypertrophy (Wang, Liao, Fukuda, Knappe, & Wu F., 2008) as well as in heart failure (Chen, Sen, Young, Wang, & Moravec, 2010 and Dong, Chen, Yang, He, & Liu, 2010). Identification of corin as the long-sought pro-ANP convertase provides important insights into the biochemical mechanism underlying natriuretic peptide processing, we now know that corin acts not only in the heart, but also in many other tissues including the kidney (Zhou, & Wu . 2014).

In the current study, an increase in corin level was significantly higher in CKD patients compared to controls. This result is in agreement with that of Fang et al., (2013), they concluded serum corin levels increase in CKD patients because CKD had markedly reduced urinary corin levels and this reduction correlated with disease severity. The renal corin mRNA and protein levels were significantly lower in CKD patients than non-CKD controls. The results indicate corin in urine due to that renal tubular corin shed into urine and that urinary and renal corin levels were reduced in CKD patients because failure in difficulties filtration corin by kidney among CKD patients and that increase serum corin levels and decrease corin in urine may reflect the underlying pathology in CKD. As a physiological activator of natriuretic peptides, increased corin indicates a high rate of active natriuretic peptides production, CKD patients required high levels of corin, in order to increase the availability of active forms of BNP, as an extreme attempt to defend against hypertension and fluid overload, through vasodilatation and natriuresis, volume overload and absence of residual renal function determine a continue secretion of corin as compensatory mechanism to increase active natriuretic peptide levels. (Ricciardi et al., 2016). In this study, we

excluded participants who had a history of coronary heart disease, tumors, diabetes, and hypertension to reduce the effects on corin level.

Polzin et al., (2010) studied decreased renal corin expression contributes to sodium retention in proteinuric kidney diseases by immunohistochemical analysis and them concluded corin might be involved in the salt retention seen in glomerular diseases because kidneys from corin knockout had increased amounts of renal β-epithelial sodium channel (β-ENaC) and its activators, phosphodiesterase (PDE) 5 and protein kinase G II. This result agree with our result by decrease sodium in serum CKD patients.

Normal human heart and kidney cells displayed the presence of corin, especially in cells around the vasculature. Both corin and $proBNP_{1–108}$ were present in the plasma of healthy human subjects and support the concept that proBNP1–108 may be processed outside of the heart in the circulation where the proprotein convertase is present (Ichiki et al., 2011). So, this study and our results can conclude impaired in real function in CKD will cause increase secretion corin from kidney cells to help in salt retention.

Another study by Wang et al., (2012) examined the role of corin in regulating blood pressure and sodium homeostasis upon dietary salt challenge and they concluded the lack of corin in mice impairs their adaptive renal response to high dietary salt and they suggested that corin deficiency may represent an important mechanism underlying salt-sensitive hypertension and the reduction was associated with sodium retention, indicating that corin defects may impair sodium homeostasis in nephrotic syndrome (Polzin et al., 2010; Klein et al., 2010, & Wong, P.C., Guo, & Zhang, 2017).

As indicated in the study by Ricciardi et al., (2016) that studied Salt–water imbalance and fluid overload in hemodialysis patients: a pivotal role of corin and illustrated corin levels in uremic patients were higher than in healthy subject. Moreover, its concentration did not change after a single hemodialysis patient's session. They concluded corin might be implicated in the regulation of salt and water balance and the disturbances of volume homeostasis of hemodialysis patients.

5.5 Total Protein, Albumin, Cholesterol and Triglyceride among The Study Population.

Without protein, our bodies would be unable to heal from injury, stop bleeding or fight infection. That's why eating protein is so important to staying healthy. The average person needs between 40 to 65 grams of protein each day (Wu G. et al., 2016). In the general, low plasma concentrations of protein are associated with CKD (Wilson, D'Agostino, Levy, Belanger, & Silbershatz, 1998). There seems to be a decrease gradual of protein in patients with CKD due to filtration of protein in urine (proteinemia) that can occur in various forms and at different levels of severity CKD and classified on the basis of the amount of protein (nephrotic or non-nephrotic), the type of protein (albuminuria or low molecular weight proteinuria), or the underlying pathological damage (glomerular vs. non-glomerular). Most cases of proteinuria can be classified as tubular, overflow, or glomerular (Yamamoto, Koike, Asanuma, Takagi, & Trejo, 2016). This study provides statistical elucidation of decrease protein concentration in CKD patients compared to controls, this agrees with other studied reports and then concluded decrease protein because failure in reabsorbing protein by the kidney (Shinaberger, Greenland, Kopple, Van Wyck, & Mehrotra, 2008 and Garneata, Stancu, Luca., Stefan & Mircescu., 2016).

Albumin considers low molecular weight and simple protein compare to other proteins in serum so it can be filtration rapidly by the kidney in urine among CKD patients and causes hypoalbuminemia (Ma, Zuo, Chen, Luo, Yu, Li, & Xu, 2006 and Suchy-Dicey, Laha, Hoofnagle, Newitt, Sirich, Meyer, & Thummel, 2015). Results of this study shown no significant different between CDK patients and controls for serum albumin that agreement with others studied by Muhaisen et al., (2012), & Chuang, Liao, Hung, Chou Y.C., & Chou P., (2017) and reported serum albumin is normal in CKD patients because they treatment with albumin (Choi, Kim, Y., Kim, S.M., Shin, J., & Jang, 2012) and also albumin treated recommended in Ministry of health protocols in Gaza strips.

Dyslipidemia with elevated cholesterol, triglyceride concentration is common in nephrotic syndrome but is not a typical feature of patients with advanced CKD (Peev, Nayer, & Contreras, 2013). Our study showed a significant increase in total cholesterol,

triglycerides in CKD patients as compared to the controls. This finding is in agreement with other studies that assessed the association between CKD with dyslipidemia (Mikolasevic, Žutelija, Mavrinac, & Orlic, 2017). Studies conducted on older patients in different stages of CKD found that CKD patients had a high prevalence of dyslipidemia (Shoji, Matsuo, Egusa, Yamasaki, & Kashihara, 2012 & Vaziri, 2014). Dyslipidaemia is associated with rapid decline in renal function in CKD patients. This mechanism is unknown, but it has been postulated that mesangial cells bind and take up oxidized LDL which then causes injury to mesangial, epithelial and endothelial cells by favouring recruitment of inflammatory cells such as macrophages which release cytokines, chemokines and growth factors. Dyslipidemia may cause vascular endothelial cell injury, leading to an increase in neutral endopeptidase release, thus increasing the degradation of BNP, so that required high levels of corin. (Adejumo, Okaka & Ojogwu, 2016).

5.6 Electrolytes among Study Population

Electrolytes are minerals found in body fluids that carry an electric charge and are essential to keeping the heart, nerves, and muscles functioning properly. One of the major roles of electrolytes is to ensure that fluid levels inside and outside the cell are balanced (Firsov, Tokonami, & Bonny, 2012). The cell can adjust its fluid levels by changing the concentration of electrolytes. The function of electrolytes is sustaining of the osmotic gradient which is essential for nerve and muscle function, hydration, and maintaining blood pH levels. Additionally, electrolytes carry electrical impulses across the cell and to neighboring cells in order to promote muscle contractions and nerve impulses. The most common electrolytes found in the body are calcium (Ca), sodium (Na), potassium (K), phosphate (Ph), and chloride (CL). The kidneys play an important role in ensuring that electrolyte levels remain invariant despite any changes the body may undergo. Having an excess or an insufficiency of electrolytes in the body can be dangerous and in some cases fatal (Alcázar, 2007 & Habbu, Sugoor, and Kale, 2014).
Patients included in the study showed significantly lower sodium and total calcium in CKD patients compared to the controls. In contrast, there was no statistically significant difference between patients and controls with regard to the means of potassium, phosphate, and chloride, since there were nearly equal means of controls and patients.

The results of the present study are in accordance with other study carried out on patients with CKD. It showed that serum decrease sodium and total calcium in CKD patients compared to the controls no statistically significant difference for potassium, phosphate, and chloride between patients and controls (Martín–Llahí, Guevara, Torre, Fagundes, & Restuccia, 2011, and Hering, Mahfoud, Walton, Krum, & Lambert, 2012 Palmer & Clegg, 2015 and Ricciardi et al., 2016). In CKD patients the kidneys fail to excrete the phosphorus, When GFR falls, the phosphorus clearance decreases, leading to phosphorus retention. The BNP plays an important role in regulating blood volume, and water balance, also BNP has been recognized to be one of the protective mediators against the deleterious effects of prolonged activation of the renin-angiotensin-aldosterone system, partly due to its inhibitory actions on renin or aldosterone release (Hruska, Mathew, Lund, Qiu, & Pratt, 2008).

Electrolyte imbalance among CKD patient will be risk factors for many complications such as irregular heartbeat, fast heart rate (cardiovascular disease), fatigue, lethargy, convulsions or seizures, nausea, vomiting, diarrhea or constipation, abdominal cramping, muscle weakness, muscle cramping , irritability, confusion, headaches, hypertensions (Armstrong, 2013 and Morelock, 2015).

5.7 Correlation between Serum Corin Levels and Studied Parameters

On the light of the present results, serum corin level has a significant inverse relation with age, height, weight, BMI, total protein, calcium, and GFR. This result agrees with Ricciardi et al., 2016 and Rame, Tam, McNamara, Worcel, & Sabolinski, 2009, they found serum corin levels have a negative correlation with age, height, weight, BMI, and GFR. In contrast, Ichiki et al., (2011) and Peleg et al., (2013) found a positive correlation between corin and age. In the other hand, Peleg et al., (2013) found a weak significant correlation of corin to age and Fathy et al., (2015) reported serum corin no correlation with age and BMI. The variation in our and researches results because all target groups were adults in the previous study but in our study target groups were children male and this first study assess level corin among children.

Cleary, Ricciardi et al., (2016) demonstrated that corin might be implicated in the regulation of salt homeostasis. This result was in line with this study that shown a negative association between serum calcium and corin.

The present study demonstrated significantly serum corin levels have positive correlation with urea and phosphorus, our results indicate an increase in corin levels secretion by kidney uncontrolled in CKD and that explain by unbalance in hormones secretion by kidney that also affected on other hormones such as parathyroid hormones (PTH) which associated with raised blood urea levels (Vhora, Munde, Bale, & Kakrani, 2015). The association between Natriuretic peptides NP levels and renal function is complex (Armaly et al,. 2013). CKD Patients with reduced renal function accounts for one component of the increase in NP levels, and so elevated of corin. The majority of studies found a significant interrelationship between serum corin and phosphorus. On the other hand, this studied suggested a role in bone differentiation or remodeling, acting on mesenchymal stem cells (Liu, Martina, Hutmacher, Hui, & Leen, 2007). Moreover, low serum corin levels have been detected in patients with osteopenia and osteoporosis, confirming a potential role of corin in regulating bone metabolism (Zhou, Zhu, Liu, Fang, & Wu, 2013).

Finally, no significant correlation between serum corin levels and duration CKD, triglyceride, SBP, creatinine, sodium, potassium, chloride, albumin & cholesterol and theses were reported that serum corin levels not significantly associated with these parameters by other studies (Chan, Knudson, Wu, Morser, & Dole, 2005 and Armaly et al., 2013). In contrast, some researcher reported this parameter was a significant correlation with serum corin (Fang et al 2013 and Peng, Zhang, Cai, Liu, & Ding et al 2015). The reason for these conflicting results may be due to the small number of the study population.

Chapter 6

Conclusion and Recommendation

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6.1 Conclusion

1-This is the first study performing to evaluate the association between serum corin and CKD patients. We found that serum corin was significantly increased in patients with CKD compared with healthy controls.

2- Our findings suggest that serum soluble corin may be a risk factor or a biomarker of CKD.

3- CKD patients require high levels of corin, in order to increase the availability of active forms of BNP.

4- The results indicate that corin expression in the kidney may represent an underlying pathological change in CKD patients.

5- The mean levels of blood urea, creatinine and uric acid were significantly increased in cases compared to controls.

6- The mean levels of triglycerides and cholesterol were significantly higher in cases compared to controls.

7- Serum corin levels showed clearly significant positive correlations with onset of CKD, urea, and phosphorus.

6.2 Recommendations

1. Introducing of corin test for CKD patients in Gaza hospitals is recommended.
2. Monitoring of serum corin levels as predisposing factor in CKD patients.
3. These findings are expected to stimulate more studies to understand the biology of corin and its role in CKD to avoid and manage renal complications.

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Appendices

Appendix 1: Questionnaire

أخي المواطن الكريم/ أرجو مساعدتنا في إتمام هذه الدراسة و التي تختص في تقييم مستوى إنزيم الكورين لدى مرضى الكلى المزمن من الأطفال الذكور في قطاع غزة والتي هدفها المساعدة في الوقوف على مسبباته وذلك للحد من مضاعفاته

Personal data			
Name: ……			
Tel. No.: ……			
Age (years): ……..			
Education (years)			
BMI:	Weight: Kg	Height: cm	
Employment:	□ Yes	□ No	
Family income per month (NIS):	□<1000 □ 1000-2000	□ >3000	
Systolic BP:	Diastolic BP:		
Clinical data			
Age at diagnosis KD (years): …… Duration of KD (years): ……			
Do you have:			
Family history of renal failure		□ Yes	□ No
Diabetes		□ Yes	□ No
Hypertension		□ Yes	□ No
Retinopathy		□ Yes	□ No
Neuropathy		□ Yes	□ No
Cardiovascular diseases		□ Yes	□ No
Recurrent infections		□ Yes	□ No
Type of drugs ……..			

أنا موافق على تعبئة هذا الاستبيان الذي يتعلق بصحة طفلي

التوقيع: ..... التاريخ: .....

شكرا لكم على حسن تعاونكم

الباحثة/ هناء مهنا

Appendix 2: Helsinki Committee

المجلس الفلسطيني للبحث الصحي
Palestinian Health Research Council
تعزيز النظام الصحي الفلسطيني من خلال مأسسة استخدام المعلومات البحثية في صنع القرار
Developing the Palestinian health system through institutionalizing the use of information in decision making

Helsinki Committee
For Ethical Approval

Date: 2016/12/05 **Number:** PHRC/HC/181/16

Name: HANAA M. MUHANNA الاسم:

We would like to inform you that the committee had discussed the proposal of your study about:

نفيدكم علماً بأن اللجنة قد ناقشت مقترح دراستكم حول:

Corin Status and Some Biochemical Parameters among Chronic Kidney Diseased Male Children in Gaza Strip

The committee has decided to approve the above mentioned research. Approval number PHRC/HC/181/16 in its meeting on 2016/12/05

و قد قررت الموافقة على البحث المذكور عاليه بالرقم والتاريخ المذكوران عاليه

Signature

Member 5/12/2016

Chairman 5/12/2016

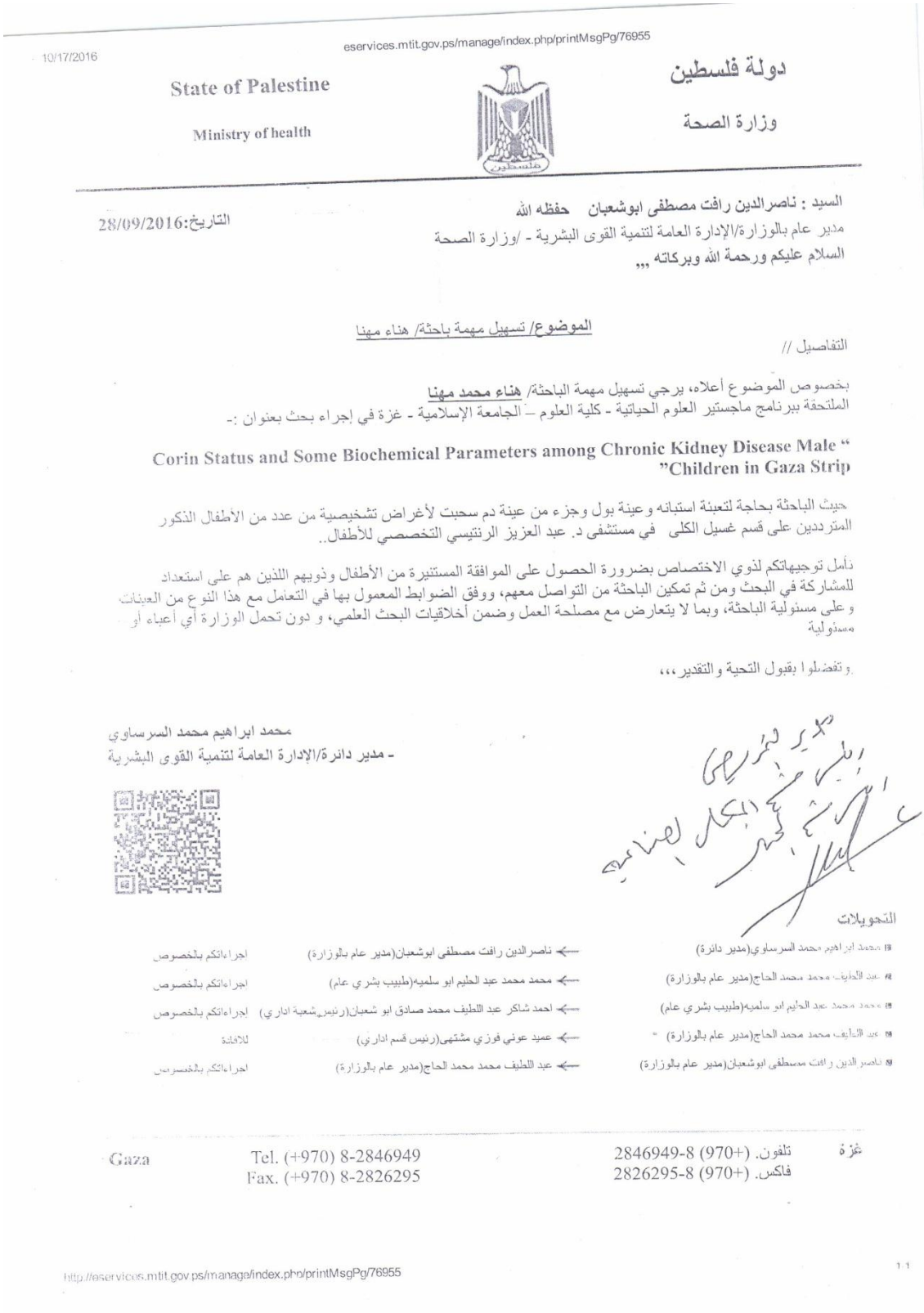
Member 5/12/2016

Genral Conditions:-
1. Valid for 2 years from the date of approval.
2. It is necessary to notify the committee of any change in the approved study protocol.
3. The committee appreciates receiving a copy of your final research when completed.

Specific Conditions:-

E-Mail:pal.phrc@gmail.com
Gaza - Palestine غزة - فلسطين
شارع النصر - مفترق الجوزة

Appendix 3: Permission Letter



10/17/2016 eservices.mtit.gov.ps/manage/index.php/printMsgPg/76955

State of Palestine
Ministry of health

دولة فلسطين
وزارة الصحة

التاريخ:28/09/2016

السيد : ناصرالدين رافت مصطفى ابوشعبان حفظه الله
مدير عام بالوزارة/الإدارة العامة لتنمية القوى البشرية - /وزارة الصحة
السلام عليكم ورحمة الله وبركاته ,,,

الموضوع/ تسهيل مهمة باحثة/ هناء مهنا

التفاصيل //

بخصوص الموضوع أعلاه، يرجي تسهيل مهمة الباحثة/ هناء محمد مهنا
الملتحقة ببرنامج ماجستير العلوم الحياتية - كلية العلوم – الجامعة الإسلامية - غزة في إجراء بحث بعنوان :-

"Corin Status and Some Biochemical Parameters among Chronic Kidney Disease Male Children in Gaza Strip"

حيث الباحثة بحاجة لتعبئة استبانه و عينة بول وجزء من عينة دم سحبت لأغراض تشخيصية من عدد من الأطفال الذكور المترددين على قسم غسيل الكلى في مستشفى د. عبد العزيز الرنتيسي التخصصي للأطفال..

نأمل توجيهاتكم لذوي الاختصاص بضرورة الحصول على الموافقة المستنيرة من الأطفال وذويهم اللذين هم على استعداد للمشاركة في البحث ومن ثم تمكين الباحثة من التواصل معهم، ووفق الضوابط المعمول بها في التعامل مع هذا النوع من العينات و على مسئولية الباحثة، وبما لا يتعارض مع مصلحة العمل وضمن أخلاقيات البحث العلمي، و دون تحمل الوزارة أي أعباء أو مسئولية

و تفضلوا بقبول التحية و التقدير,,,

محمد ابراهيم محمد السرساوي
- مدير دائرة/الإدارة العامة لتنمية القوى البشرية

التحويلات

محمد ابراهيم محمد السرساوي(مدير دائرة)	ناصرالدين رافت مصطفى ابوشعبان(مدير عام بالوزارة)	اجراءاتكم بالخصوص
عبد اللطيف محمد محمد الحاج(مدير عام بالوزارة)	محمد محمد عبد الحليم ابو سلميه(طبيب بشري عام)	اجراءاتكم بالخصوص
محمد محمد عبد الحليم ابو سلميه(طبيب بشري عام)	احمد شاكر عبد اللطيف محمد صادق ابو شعبان(رئيس شعبة اداري)	اجراءاتكم بالخصوص
عبد اللطيف محمد محمد الحاج(مدير عام بالوزارة)	عميد عوني فوزي مشتهى(رئيس قسم اداري)	للافادة
ناصر الدين رافت مصطفى ابوشعبان(مدير عام بالوزارة)	عبد اللطيف محمد محمد الحاج(مدير عام بالوزارة)	اجراءاتكم بالخصوص

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