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Serum Vitamin D Level in Chronic Kidney Disease Patients from Gaza Strip

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Date: التاريخ: 28 / 11 / 2015

The Islamic University of Gaza

Deanship of Post graduate Studies

Biological Sciences Master Program

Serum Vitamin D Level in Chronic Kidney Disease Patients from Gaza Strip

Submitted in Partial Fulfillment for the Master Degree of Science in Biotechnology

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June, 2015

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الجامعة الإسلامية – غزة
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مكتب نائب الرئيس للبحث العلمي والدراسات العليا هاتف داخلي 1150

الرقم... ج.س.غ/35/..... Ref
التاريخ 2015/08/31 Date.....

نتيجة الحكم على أطروحة ماجستير

بناءً على موافقة شئون البحث العلمي والدراسات العليا بالجامعة الإسلامية بغزة على تشكيل لجنة الحكم على أطروحة الباحثة/ سُها عادل محمد عبدالله لنيل درجة الماجستير في كلية العلوم قسم العلوم الحياتية – تحاليل طبية وموضوعها:

مستوى فيتامين D عند مرضى اعتلال الكلى في قطاع غزة

Serum Vitamin D Level in Chronic Kidney Disease Patients from Gaza Strip

وبعد المناقشة العلنية التي تمت اليوم الاثنين 16 ذو القعدة 1436هـ، الموافق 2015/08/31م الساعة الثانية عشرة ظهراً بمبنى 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 تسخر علمها في خدمة دينها ووطنها.

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Soha	**Soha Adel Abdallah**	**June, 2015**

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Dedication

I dedicate this work to:

My beloved parents who have always supported me

My Husband who encouraged me to accomplish this thesis

to my brothers

All researchers who are working to improve the quality of life

Dedication is almost expressed to the Palestinian people who have suffered and are struggling with the persistence to have a free Palestine.

Soha Adel Abdallah

Acknowledgment

I would like to express my deepest gratitude and appreciation to my supervisor **Prof. Dr Maged M. Yassin,** Professor of Physiology, Faculty of Medicine, The Islamic University of Gaza for his planning and initiating of this work and for his continuous support, encouragement and kind of supervision that leads to the emergence of this work in its current form.

Special thanks for the dearest persons to me my **mother**, my **father** and my Husband **Dr.Alaa** for their support and encouragements.

I would like to thank the staff of kidney disease unit in AL-Shifa hospital and Nasser Medical Complex for their facilitation and helping me in samples collection.

My special thanks to **Mr. Abed EL- Rahman Hamad** for his help in statistical analysis.

At the end, I am very grateful to every person who participated and helped me to complete this study.

Serum vitamin D level in chronic kidney disease patients from Gaza Strip

Abstract

Background: chronic kidney disease (CKD) is one of the leading cause of death among the Palestinians. Although vitamin D deficiency has been recently linked to CKD, biochemical tests in Gaza hospitals and clinics are restricted to monitoring kidney function by routine tests. Therefore, introducing vitamin D test for CKD patients may be helpful in understanding patient's condition and help in disease management.

Objective: To assess serum vitamin D level in CKD patients from Gaza Strip.

Material and methods: This case-control study comprised 42 CKD patients and 42 healthy controls. Patients were taken from Kidney unit in Al-Shifa hospital and Nasser Medical Complex in Gaza Strip. Controls were selected from the general population. Questionnaire interview was applied. Serum vitamin D, urea, creatinine, uric acid, glomerular filtration rate (GFR), total protein, albumin, globulin, calcium and phosphorus were determined. Data were analysed using SPSS version 18.0.

Results: The mean ages of cases and controls were 55.3±8.6 and 54.9±8.2 years, respectively. CKD was more frequent among unemployed individuals, families with low income as well as among families with history of CKD ($P<0.05$). The mean levels of vitamin D were significantly lower in cases compared to controls (29.7±12.9 *versus* 35.2±9.9 ng/dl, P=0.033). Serum urea, creatinine and uric acid were found to be significantly higher in cases (84.6±47.4 and 1.90±1.20 and 7.92±2.29 mg/dl, respectively) compared to controls (35.7± 13.5 and 0.81±0.27 and 5.18±2.31 mg/dl) with P=0.000. In contrast, The mean value of GFR was significantly declined in cases compared to controls (62.4±32.5 *versus* 124.6±45.4, P=0.000). There was a significant decrease in serum total protein and albumin in cases compared to controls (7.0±0.5 and 5.2±0.40 *versus* 7.3±0.6 and 5.4±0.59, P=0.005 and P=0.023, respectively). Serum calcium was significantly declined in cases compared to controls, whereas serum phosphorus showed none significant increase in cases (8.61±0.77 *versus 9.12±0.69*, P=0.003 and 4.72±0.94

versus 4.49±0.85, P=0.239, respectively). Vitamin D level was found to be significantly lower in individuals with family history of CKD (P=0.038). Vitamin D levels were positively correlated with GFR (r=0.258, P=0.020), total protein (r=0.283, P=0.011), albumin (r=0.278, P=0.012), globulin (r=0.159, P=0.156) and calcium (r=0.562, P=0.001) and negatively correlated with urea (r=-0.302, P=0.005), creatinine (r=-0.343, P=0.001), uric acid (r=-0.249, P=0.022) and phosphorus (r=-0.168, P=0.125).

Conclusions: Serum vitamin D levels were significantly lower in CKD patients compared to controls. Vitamin D levels were lower in individuals with family history of CKD. Vitamin D levels were positively correlated with GFR, total protein, albumin, globulin and calcium, and negatively correlated with urea, creatinine, uric acid and phosphorous.

Keywords: Chronic kidney disease, Vitamin D, Gaza strip.

مستوى فيتامين د في مصل الدم لمرضى الكلى المزمن في قطاع غزة

ملخص الدراسة

المقدمة: تعد امراض الكلى احد اسباب الوفاة بين الفلسطينيين. وعلى الرغم من ارتباط نقص فيتامين د بقوة بمرض الكلى المزمن، الا ان الفحوصات التى تعمل لهذا المرض محدودة. لذلك ادخال اختبار فيتامين د في مستشفيات غزة لمرضى الكلى يساعد في فهم حالة المريض وتساعد في ادارة المرض.

الهدف: تقييم مستوى فيتامين د في امراض الكلى المزمنة في قطاع غزة.

الطرق والادوات: منهج الدراسة (مجموعة مرضية-مجموعة ضابطة) المجموعة المرضية تحتوى على 42 مريض كلى (21رجل- 21امراة)، 42 شخصا من الاصحاء (21 رجل- 21امراة). الحالات تم اخذها من مستشفى الشفاء و مجمع ناصر الطبى، اما الاصحاء تم اخذهم من المجتمع. وقد تم الحصول على النتائج من خلال المقابلة الشخصية للمرضى والاصحاء، وتم قياس مستوى فيتامين د، اليوريا، الكرياتينين، حمض اليوريك ، معدل الترشيح الكبيبى، والبروتين والالبيومين والكالسيوم والفوسفور، وقد تم تحليل البيانات والنتائج باستخدام البرنامج الاحصائى SPSS-18.

النتائج: تبين من الدراسة ان متوسط الاعمار حالات والضوابط هو 8.6±55.3 و 8.2±54.9 سنة على التوالى، وان مرض الكلى المزمن اكثر شيوعا بين الغير عاملين وذوى الدخل المنخفض والافراد الذين لديهم تاريخ عائلى في مرض الكلى. كان متوسط مستوى فيتامين د في الحالات اقل بكثير مقارنة مع الضوابط. مستويات اليوريا والكرياتينين وحمض اليوريك كانت مرتفعة بشكل ملحوظ في الحالات مقارنة مع الضوابط. كان مستوى معدل الترشيح الكبيبىGFR منخفض في الحالات مقارنة مع الضوابط. وقد انخفض مستوى البروتين والالبيومين في الدم بشكل ملحوظ في الحالات مقارنة مع الضوابط. كما انخفض معدل الكالسيوم وارتفع الفوسفور.

واظهرت النتائج وجود علاقة ايجابية بين مستوى فيتامين د مع مستوى معدل الترشيح الكبيبى والبروتين والالبيومين والجلوبيولين والكالسيوم، وعلاقة سلبية مع كل من اليوريا والكرياتينين وحمض اليوريك والفوسفور.

الكلمات المفتاحية: مرضى الكلى المزمن، فيتامين د، قطاع غزة.

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Chapter 1

Introduction

1.1 Overview

Chronic kidney disease (CKD) is a modern day global epidemic and it is now recognized as a public health issue. Chronic kidney disease is defined as either kidney damage or glomerular filtration rate (GFR) <60 mL/min/1.73 m^2 for >3 months or more, irrespective of cause **(Levey et al., 2005; Eckardt et al., 2009 and Levey et al., 2011).** Kidney damage in many kidney diseases can be ascertained by the presence of albuminuria **(Guh, 2010)**. The severity of CKD is classified into five stages according to the level of GFR, with stage 1 (GFR≥90 ml/min/1.73m^2) being the mildest and usually causing few symptoms and stage 5 (GFR<15 ml/min/1.73m^2 or dialysis) being a severe illness with poor life expectancy if untreated **(National Kidney Foundation, NKF, 2002; Bauer et al., 2008 and Levey and Coresh, 2012).**

Most epidemiological information on CKD originates from data available on end stage renal disease (ESRD). Little information is available on the prevalence of earlier stages of CKD, as patients are often asymptomatic **(Warady and Chadha, 2007).** Centers for Disease Control and Prevention (CDC) have estimated that more than 10% of adults in USA, more than 20 million people, may have CKD **(CDC, 2014).** United Kingdom estimates suggested that the prevalence of CKD was 8.5% **(Stevens et al., 2007).** In Egypt the prevalence of CKD was estimated at 10.6% **(Gouda et al., 2011).** In Jordan, ESRD accounted for around 3% of the population **(The Hashemite kingdom of Jordan, Ministry of Health, 2008).** Renal failure is the 6th leading cause of death in Palestine **(Ministry of Health, MOH, 2010).** The total prevalence of ESRD among Palestinian patients in West Bank was 240.3 per million population **(Khader et al., 2013).**

The risk factors for developing CKD include diabetes mellitus, hypertension, obesity, cardiovascular disease, family history of CKD and age over 60 years **(Stenvinkel, 2010; Huda et al., 2012 and CDC, 2014)**. Most people may not

have any severe symptoms until their kidney disease is advanced. However, the symptoms of chronic kidney disease may include tiredness, troubles in concentration, poor appetite, troubles in sleeping, muscle cramping at night, swollen feet and ankles, puffiness around eyes especially in the morning, have dry and itchy skin, and need to urinate more often especially at night **(Wiggins and Johnson, 2012 and NKF, 2013).**

Vitamin D is a fat-soluble vitamin that plays an essential role in calcium homeostasis and the maintenance of normal function in multiple tissues. Humans obtain vitamin D either directly from the diet or through exposure to solar ultraviolet B radiation **(Holick, 2011 and Kannan and Lim, 2014).** In addition to its well-recognized effects on skeletal health, vitamin D has suggested to have a potential role in other disease states and health conditions including chronic kidney disease, cardiovascular disease, type 2 diabetes, autoimmune disorders and cancer **(Drake et al., 2010; Dalgard et al., 2011; Joergensen et al., 2012; Satirapoj et al., 2013 and Nigwekar et al., 2014).**

Recent studies have reported that vitamin D deficiency or insufficiency is common in patients with CKD, and serum levels of vitamin D appear to have an inverse correlation with kidney disease **(Adams and Hewison, 2010; Dusso et al., 2011; Nigwekar et al., 2012; Rozita et al., 2013; Kim and Kim, 2014 and Obi et al., 2015).** In this regard, it has been shown that treatment with active vitamin D or its analogues can ameliorate renal injury **(Rojas-Rivera et al., 2010 and Kim and Kim, 2014).** In addition, impaired vitamin D metabolism in CKD was addressed **(Bosworth and de Boer, 2012).**

In Gaza strip, studies on vitamin D status in patients with chronic disease are limited. Only two studies related vitamin D to coronary artery disease (CAD) and type 2 diabetes in Gaza strip have been recently emerged **(Elhenawe, 2014 and Masoud, 2014).** No previous study linked vitamin D to CKD. Therefore, the present study is the first to assess serum vitamin D level in CKD patients from Gaza strip.

1.2 General objective

To assess serum vitamin D level in CKD patients from Gaza strip.

1.3 Specific objectives

1. To determine vitamin D level in cases compared with controls.
2. To estimate kidney function through determination of urea, creatinine ,uric acid and GFR in cases and controls.
3. To test serum total protein, albumin and globulin in cases in comparison with controls
4. To evaluate serum calcium and phosphorus in cases and controls
5. To verify the relationship between vitamin D and the previous studied parameters**.**

1.4 Significance

1. Chronic kidney disease is being increased worldwide. In Palestine, renal failure is the 6th leading cause of death **(National Health Service, NHS, 2010).**

2. In Gaza Strip only three studies have been investigated vitamin D status in patients with chronic disease **(Elhenawe, 2014, Masoud, 2014 and Elhamalawy, 2015).** To my best knowledge, no previous study linked vitamin D to CKD. Therefore, as far as I know this is the first study to assess serum vitamin D level in CKD patients in Gaza Strip.

3. Treatment of CKD patients with vitamin D could be useful in the disease management strategy aiming to long delay the development of renal failure.

Chapter 2

Literature Review

2.1 The kidneys

2.1.1 Location and structure

The kidneys are small, dark red organs lie against the dorsal body wall beneath the parietal peritoneum in superior lumbar region where they receive some protection from the lower part of the rib cage **(Figure 2.1)**. An adult kidney (about 12 cm long, 6 cm wide, and 3 cm thick) has a medial indentation (the hilus) in which there are renal artery, renal vein, and ureter **(Marieb, 2003).** The kidney has three regions, outer granulated layer called renal cortex, renal medulla that consists of cone shaped tissue masses called medullary pyramids, and renal pelvis which is a central space or cavity that is continuous with the ureter **(Mader, 2004).**

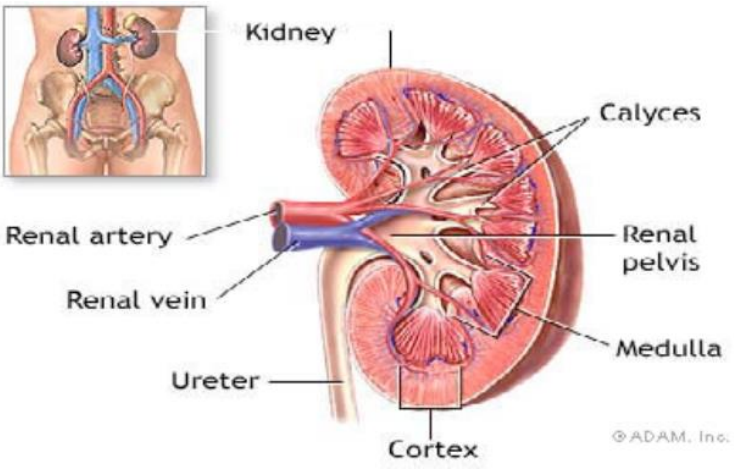


Figure 2.1. Location and structure of the kidney **(Marieb, 2003)**

Each kidney contains approximately one million tiny structures called nephrons **(Figure 2.2).** Nephrons are responsible for the processes of filtration, reabsorption, and secretion that go on in the kidney to form the urine product **(Marieb, 2003).** The nephron consists of two main 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 deliver the final urine product into the calyces and pelvis of the kidney **(Thibodeau and Patton, 1999; Mader, 2004 and Guyton and Hall, 2011).**

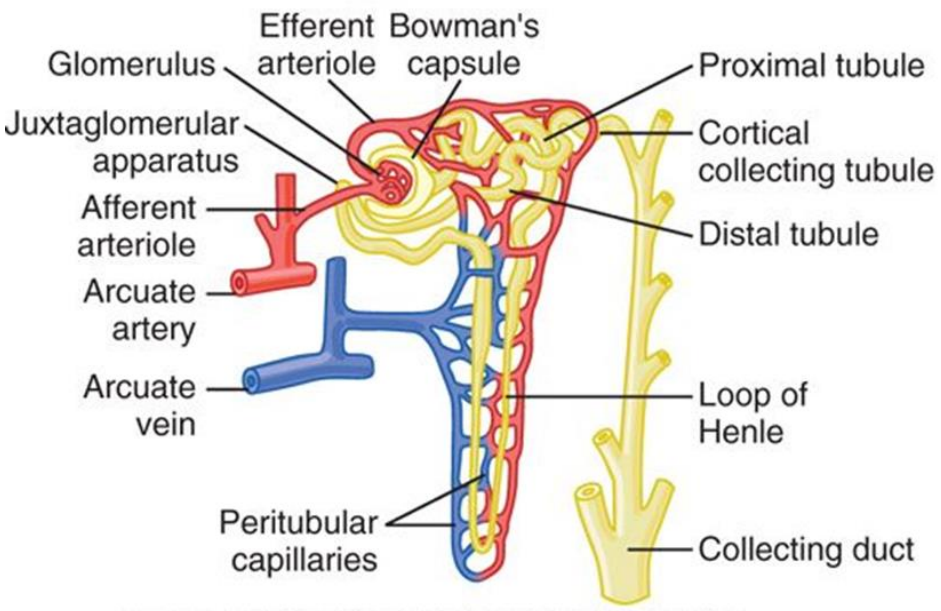


Figure 2.2. Structure of the nephron **(Guyton and Hall, 2011)**

2.1.2 Functions of the kidney

The kidneys excrete natural waste products, including urea and creatinine, as well as foreign substances like alcohol and drugs, from the body. The kidneys also play a role in water level balancing and acid regulation **(Faratro et al., 2004).** The kidneys also produce and secrete important hormones including renin, erythropoietin and vitamin D. Renin is involved in regulating blood pressure, erythropoietin is used to stimulate the bone marrow to produce red blood cells, and vitamin D is needed to absorb the calcium from food in the intestine **(Faratro et al., 2004 and Barret et al., 2010).**

2.2 Chronic kidney disease

2.2.1 Definition

The National Kidney Foundation (2002) defines CKD as ″kidney damage for ≥ 3 months, as confirmed 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 ascertained by either kidney biopsy or markers of kidney damage, such as urine abnormalities (proteinuria), blood abnormalities or abnormalities on imaging studies.

Levey et al. (2005) defined CKD as kidney damage or GFR <60 ml/min/1.73 m^2 for 3 months or more, irrespective of cause. Kidney damage in many kidney diseases can be ascertained by the presence of albuminuria, defined as albumin-to-creatinine ratio >30 mg/g in two of three spot urine specimens. Glomerular filtration rate can be estimated from calibrated serum creatinine and estimating equations, such as the modification of diet in renal disease study equation or the Cockcroft-Gault formula (table 2.1).

Chronic kidney disease is defined according to a decrease in the GFR and kidney damage such as proteinuria (>200 mg/day or protein to creatinine ratio (PCR) >200 mg/g creatinine) or albuminuria (urinary albumin excretion (UAE) ≥ 30mg/day or albumin to creatinine ratio (ACR) ≥ 30mg/g creatinine) **(Guh, 2010).**

International Society of Nephrology (2013) defined CKD as abnormalities of kidney structure or function, present for >3 months, with implications for health. Markers of kidney damage (one or more) include: Albuminuria (ACR≥30 mg/g), urine sediment abnormalities, electrolyte and other abnormalities due to tubular disorders, abnormalities detected by histology, structural abnormalities detected by imaging, history of kidney transplantation and GFR <60 ml/min/1.73m^2.

2.2.2. Glomerular filtration rate

Glomerular filtration rate describes the flow rate of filtered fluid through the kidney. Glomerular filtration rate provides an excellent measure of the filtering capacity of the kidneys. A low or decreasing GFR is a good index of CKD **(Gansevoort et al., 2011; International Society of Nephrology, 2013 and Nigwekar et al., 2014).**

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 2.1). 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 2.1 Prediction of GFR based on serum creatinine

Author's equation	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

2.2.3 Classification of chronic kidney disease

Chronic kidney disease has been classified into various 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 prevention of complications in severe CKD **(Vijayakumar et al., 2007). National Kidney Foundation (2002)** classified CKD into 5 stages according to the level of GFR (Table 2.2). For stages 1 and 2, kidney damage was assessed by spot albumin to creatinine ratio.

Table 2.2 Classification of the stages of chronic kidney disease (CKD).

CKD Stage	**Description**	**GFR (ml/min/1.73m^2)**
1	Kidney damage with normal or increased GFR	≥ 90
2	Kidney damage with mild reduction of GFR	60-89
3	Moderate reduction of GFR	30-59
4	Severe reduction of GFR	15-29
5	Kidney failure (ESRD)	<15 or dialysis

GFR: Glomerular filtration rate. ESRD: End stage renal disease.

Adopted from National Kidney Foundation **(NKF, 2002).**

2.2.4 Epidemiology of chronic kidney disease

Chronic kidney disease is an important global public health concern because of its high incidence, prevalence, morbidity, and mortality **(Levey et al., 2007).** Until recently, little has been known about the epidemiology of pre-ESRD. The main reason for this includes the absence of any systematic, population-based collection of data on CKD **(McClellan et al., 2006).** It is estimated that by 2030, over 70% of patients with ESRD will be inhabitants of developing countries, probably related to the fast rising trend of obesity and diabetes in these countries **(Barsoum, 2006 and Motlagh et al., 2009).**

Centers for Disease Control and Prevention have estimated that more than 10% of adults in USA, more than 20 million people, may have CKD **(CDC, 2014).** United Kingdom suggested that the prevalence of CKD was 8.5% **(Stevens et al., 2007).** In Canada, 1.9-2.3 million people have CKD **(Levin et al., 2007).** In Japan, the prevalence of CKD constitutes approximately 13% of the adult population **(Nakai et al., 2013)**. In addition, CKD may affect up to 7.7% of the korean population resulting in high morbidity and mortality **(Shin et al., 2014).**

The reported prevalence of CKD in Arab world is very limited. In Egypt the prevalence of CKD was estimated at 10.6% **(Gouda et al., 2011).** In Jordan, ESRD accounted for around 3% of the population **(The Hashemite kingdom of Jordan, Ministry of Health, 2008)**. In Saudi Arabia, the prevalence of CKD in the young Saudi population is around 5.7% **(Alsuwaida et al., 2010).**

The Palestinian 2005 report showed that the prevalence of renal failure was 4% with an incidence of 10.8 per 100,000, distributed as 1.1% in Gaza strip and 2.9% in the West Bank **(MOH, 2005).** Recent study showed that the total prevalence of ESRD among Palestinian patients in West Bank was 240.3 per million population **(Khader et al., 2013).**

2.2.5 Risk factors of chronic kidney disease

The most common risk factors for CKD include socioeconomic status, family history, obesity, diabetes, hypertension.

2.2.5.1 Socioeconomic status

Socioeconomic factors are known to influence both the prevalence and severity of CKD. **Zhang et al. (2012)** reported that the prevalence of CKD varied greatly between geographical regions and this might be related to variability in lifestyles and economic development. Several studies have demonstrated an increased risk of CKD in individuals of lower socioeconomic status **(Drey et al., 2003; Ward, 2008; McIntyre et al., 2011 and Abu Nada, 2012).** However, the determinants of progression of CKD are not fully understood though there are a number of proposed risk factors including socioeconomic factors for its progression **(Stringer et al., 2013).**

2.2.5.2 Family history

Genetic predisposition plays an important role in CKD. Most studies indicated that family history is a risk factor of CKD **(Levey et al., 2005; Hsu et al 2009; Baumgarten and Gehr, 2011 and CDC, 2014).** In a cohort of 1742 people participating in community-based CKD screenings, 24% reported a family history of kidney disease and 60% tested positive for microalbuminuria **(Harward et al., 2009).** In addition, the prevalence of CKD in first-degree relatives is high, estimated as three-fold higher than for a general population as reported in population studies **(Inserra et al., 2007).**

2.2.5.3 Obesity

Obesity as defined by increased body mass index (BMI) has been reported to be associated with increased risk of developing CKD **(Wang et al., 2008; Nugent et al., 2011 and CDC, 2014).** It is estimated that by 2030, over 70% of patients with ESRD will be inhabitants of developing countries, probably related to the fast rising trend of obesity in these countries **(Barsoum, 2006 and Motlagh et al., 2009).** BMI is shown to be inversely related to GFR **(Kramer, 2006 and Ishizaka et al., 2007).** In this regard, **Hyun et al. (2014)** found that fat mass gain over 5 years was independently associated with GFR decline to <60 ml/min/1.73m^2 in a relatively healthy Korean population. It was reported that adipose tissue produces a variety of hormones and proinflammatory molecules that may contribute to progressive renal damage **(Druml et al., 2010; Tesauro et al., 2011 and Wickman and Kramer, 2013).**

2.2.5.4 Diabetes mellitus

Metabolic diseases such as diabetes are known to be a risk factor of kidney injury and plays an important role in the progression of CKD. Most studies pointed out that diabetic patients have a high risk of developing CKD **(Chen et al., 2007; Nugent et al., 2011 and Maryam et al., 2012).** In addition, diabetes is one of the leading cause of CKD in the developed countries. Approximately 40% of diabetic patients had some degree of CKD in the United States **(Laliberté et al., 2009)** and 40–50% of diabetic patients had kidney injury in Japan **(Ohta et al., 2010).** In general, it is accepted that about 1 of 3 adults with diabetes has CKD **(CDC, 2014).** Diabetes mellitus is likely to cause hyperfiltration and elevation of GFR **(Cynda et al., 2004 and Yokoyama et al., 2009).**

2.2.5.5 Hypertension

Hypertension is a major risk factor for the development of CKD and progression to ESRD **(NKF, 2007; Nugent et al., 2011 and Johnson et al., 2013).** In this regard, CDC reported that approximately 1 of 5 adults with high blood pressure has CKD **(CDC, 2014).** An increase in diastolic arterial blood pressure was associated with slightly reduced GFR in the middle-aged healthy general population. This may be consistent with the hypothesis of a renal cause of essential hypertension, but can also be explained by renal damage caused by elevated blood pressure **(Mathisen et al., 2010).** In addition, hypertension and diabetes may interact synergistically to increase the risk of CKD **(Ejerblad et al., 2006).**

2.3 Vitamin D

2.3.1 Definition and structure

Vitamin D is a seco-steroid hormone and it is critically important for the development, growth and maintenance of a healthy skeleton from birth until death **(Andersen et al., 2007).** Vitamin D has other roles in human health; it can play a role in decreasing the risk of many chronic illnesses, including chronic kidney disease, cardiovascular disease, diabetes, autoimmune diseases and cancer **(Zhang and Naughton, 2010; Nigwekar et al., 2012; Grober et al., 2013 and Nigwekar et al., 2014).** The molecular structure of vitamin D is closely allied to that of classic steroid hormones in that it has the same root cyclopentanoperhydrophenanthrene ring structure (**Figure 2.3, Wang, 2013).**

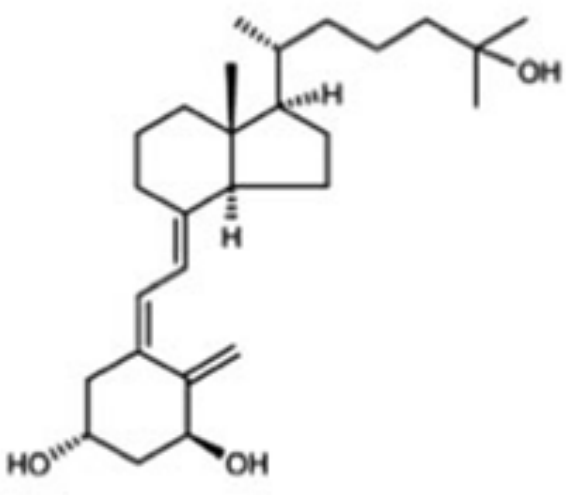


Figure 2.3. Structure of vitamin D **(Wang, 2013).**

2.3.2 Sources of Vitamin D

Vitamin D is obtained either directly from the diet or by means of photosynthesis in the skin.

1. Dietary sources of vitamin D (exogenous vitamin D)

Fish, liver oils, fatty saltwater fish, dietary products and eggs all contain vitamin D. It is also found in butter, cod liver oil, dandelion green, egg yolks, halibut, liver, milk, oatmeal, salmon, sardines, sweat potatoes, tuna and vegetables **(Balch, 2001; Palomer et al., 2008 and Holick, 2011).**

2. Photosynthesis in the skin (endogenous vitamin D)

The solar ultra violet B radiation penetrates the skin to convert 7-hydrocholesterol to previtamin D_3, which is thermodynamically unstable and undergoes thermally induced conversion to vitamin D_3 (Figure 2.4). Whatever the source, vitamin D_3 must be hydroxylated twice to produce the biologically active form. Thus, the first hydroxylation process takes place in the liver and forms 25-hydroxyvitamin D_3 and is catalysed by vitamin D-25-hydroxylase. The second hydroxylation step, which produces the final active metabolite of vitamin D_3 ($1,25(OH)_2D_3$), is mediated by 25-hydroxyvitamin D_3 1α-hydroxylase and occurs predominantly in the kidney. Then, $1,25(OH)_2D_3$ is released into the circulation where it binds to vitamin D-binding protein until it reaches its target tissue by means of the vitamin D receptors **(Chagas et al., 2012 and Kannan and Lim, 2014).**

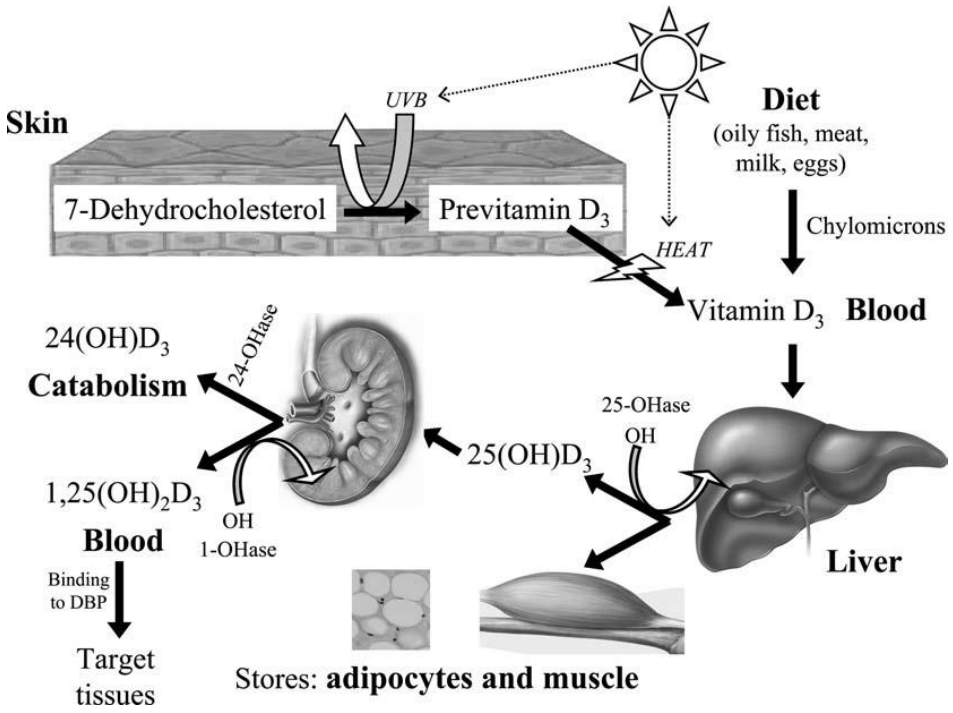


Figure 2.4 Synthesis of vitamin D. UVB: Ultra Violet B, $25(OH)D_3$: 25-hydroxyvitamin D_3, 25-OHase: Vitamin D-25-hydroxylase, 1-OHase: 25-hydroxyvitamin D_3 1α-hydroxylase, DBP: Vitamin D-binding protein (**Palomer et al., 2008**).

2.3.3. Mechanism of action of vitamin D

Vitamin D is bound to vitamin D-binding protein in circulation, crosses the cell membrane, and binds to vitamin D receptor (Figure 2.5). The conjugated vitamin D with its receptor forms a heterodimer complex with retinoid X receptor and with other factors, attaches to vitamin D–responsive elements on deoxyribonucleic acid, and alters gene expression. It has been estimated that vitamin D regulates more than 200 genes, directly or indirectly, thereby influencing a wide variety of physiological processes **(Vanga et al., 2010; Stivelman and Retnakaran, 2012 and Wang, 2013).**

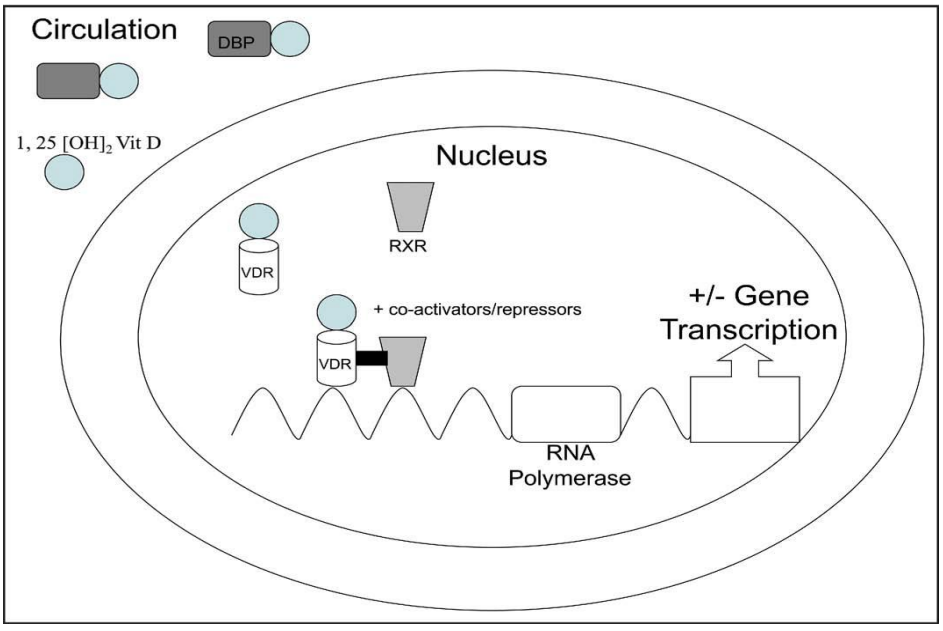


Figure 2.5 Action of vitamin D. DBP: Vitamin D-binding protein, VDR: Vitamin D receptor, RXR: Retinoid X receptor, RNA: Ribonucleic acid **(Vanga et al. 2010).**

2.3.4. Roles of vitamin D

Vitamin D has several roles in the body; many of these arise from its action on gene transcription and expression. In addition to the well-recognized effects of vitamin D on skeletal health, emerging evidence suggests a potential role for vitamin D in numerous other disease states and health conditions including chronic kidney disease, cardiovascular disease, type 2 diabetes, autoimmune disorders and cancer.

2.3.4.1 Bone health and calcium absorption

Vitamin D facilitates calcium absorption in the intestine by influencing the expression of epithelial calcium channels and thus calcium-binding proteins. This process allows calcium to be better absorbed from the foods eaten **(Holick, 2005).** Due to the increase in absorption on calcium, parathyroid hormone (PTH) levels are better regulated. When serum calcium levels are low, the parathyroid gland secretes PTH, which leads to increased production of vitamin D. This further increases absorption of calcium from the intestine as well as increases reabsorption of calcium by the kidneys**.** The third effect that increased PTH levels have on the body is that it leads to resorption of calcium from the bone in order to maintain adequate serum levels. Leaching calcium out of the matrix of bone leads to decreased bone strength. If adequate vitamin D is present before this occurs, PTH levels are likely to be kept low as calcium absorption is increased **(Wacker and Holick, 2013)**. Another way in which vitamin D works to increase bone strength is by mediating the incorporation of calcium into the matrix of bone. This strengthens the network of fibers within the bone itself thus leading to stronger bones **(Reese, 2006).**

2.3.4.2. Vitamin D and chronic kidney disease

Vitamin D deficiency is commonly observed across the spectrum of chronic kidney disease stages **(Williams et al., 2009; Holden et al., 2010; Satirapoj et al., 2013 and Obi et al., 2015).** In this regard, **Kendrick et al. (2012)** found that low plasma concentrations of vitamin D were associated with 1.2 and 1.6-fold risk of death and progression to chronic dialysis. In addition, **Pilz et al. (2011)** demonstrated a relationship of low vitamin D with mortality and cardiovascular event rate in both dialysis and pre-dialysis patients. Supplementation of vitamin D or its analogs have been shown to slow the progression of CKD **(Shoben et al., 2008; Li, 2013; Kim and Kim, 2014 and Kim et al., 2014)**. In this context, observational studies have found that CKD patients treated with active vitamin D analogs have a lower risk of death than patients who were not treated **(Kovesdy et al., 2008).** Chronic uremia, reduced kidney mass, reduced kidney function or reduced GFR, and protein losses are some of the contributors to hypovitaminosis D in CKD **(Rojas-Rivera et al., 2010).**

2.3.4.3. Vitamin D and cardiovascular disease

A growing body of evidence suggests a possible association between vitamin D deficiency and many cardiovascular disorders, including hypertension, peripheral vascular disease, CAD and heart failure. Vitamin D receptors are located on vascular smooth muscle, endothelium, and cardiomyocytes **(Wang et al., 2008).** One of the main mechanisms whereby vitamin D appears to decrease cardiovascular disease risk is its effect on hypertension through the renin-angiotensin system **(Vaidya et al., 2011 and Kienreich et al., 2013).** Most of studies reported significant inverse relations between vitamin D levels and blood pressure (**Scragg et al., 2007; Abuannai and O'Keefe., 2011 and Kienreich et al., 2013).** Vitamin D deficiency is also strongly associated with increased thickness of the intima-media in carotid arteries **(Targher et al., 2006 and Joergensen et al., 2012).** In this regarded vitamin D was found to be associated with atherosclerosis and CVD events **(Lavie et al., 2011 and Ku et al., 2013)**. Also, **Virtanen et al. (2011)** demonstrated an increased risk of CAD death in individual with vitamin D deficiency**.** In addition, **Elhenawe, (2014)** found a progressive decrease in vitamin D level with increasing the duration of CAD.

2.3.4.4. Vitamin D and type 2 diabetes

Most studies have reported a significant inverse association between serum vitamin D and the presence of type 2 diabetes **(Dalgard et al., 2011; Gonzalez-Molero et al., 2012; Djalali et al., 2013 and Mansuri et al., 2014).** In addition, a number of studies have also investigated the role of vitamin D in the primary pathophysiological disorders underlying type 2 diabetes, specifically insulin resistance and β-cell dysfunction. A significant inverse association of serum vitamin D with insulin resistance was documented **(Tracy and Mazen, 2010 and Afsaneh et al., 2013).** Regarding β-cell function, several studies reported a positive association between vitamin D and β-cell function **(Ozfirat and Chowdhury, 2010 and Takiishi et al., 2013).**

2.3.4.5. Vitamin D and autoimmune diseases

Various epidemiological studies suggested associations between vitamin D deficiency and a higher incidence of autoimmune diseases, such as type -1-diabeties, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis and inflammatory bowel disease **(Grober et al., 2013 and Sabbagh et al., 2013).** Vitamin D receptors are present in many cell types including various immune cells such as antigen-presenting-cells, T cells, B cells and monocytes (**Prietl et al., 2013**). A recent systematic review analyzed results from 219 published studies and concluded that vitamin D seems to play a beneficial role in the prevention of autoimmunity but that there is still a lack of randomized controlled clinical trials in this field (**Antico et al., 2012).**

2.3.4.6. Vitamin D and cancer

An inverse association between vitamin D and the incidence of several cancers and mortality from these cancers has been shown in case-control studies, prospective and retrospective studies (**Freedman et al., 2007; Drake et al., 2010; Manson et al., 2011; Robsahm et al., 2013 and Ying et al., 2014)**, and especially for cancers of the colon, breast and prostate **(Holick, 2007).** Several studies show that supplementation with calcium and vitamin D reduced the relative risk (RR) of cancer **(Lappe et al., 2007 and Crew, 2013).** The anti-carcinogenic effects of vitamin D could be explained by anti-proliferative effects on cancer cells by promoting cyclin-dependent kinase inhibitor synthesis, and by influencing several growth factors and their signaling pathways **(Fleet et al., 2012).** In addition, vitamin D promotes various apoptotic mechanism and cell differentiation and suppresses angiogenesis and tumor invasion and metastasis (**Deeb et al., 2007; Krishnan and Feldman, 2011; Hossein et al., 2013 and Gröber, 2014).**

2.4 Related studies

In a cross-sectional analysis of 825 consecutive incident hemodialysis patients from 569 unique centers in the United States, **Wolf et al**. **(2007)** reported that over 3/4th of the cohort was vitamin D deficient (Serum vitamin D <30 ng/ml) and about 1/5th of the cohort was severely deficient (Serum vitamin D <10 ng/ml). The authors concluded that the prevalence of vitamin D deficiency in the CKD population has been described to range between 70 and 80%.

In an observational study of 226 patients with stage III and IV CKD, **Inaguma et al. (2008)** reported that low serum vitamin D concentrations were associated with an increased risk of death. In another study of 520 male patients with stage 3-5 CKD, not yet on dialysis, treatment with oral calcitriol was associated with better survival. The incident rate ratio for mortality in patients treated with calcitriol compared to untreated patients was 0.35 (95% CI, 0.23-0.54; p<0.001) and for the combined endpoint of death and dialysis initiation was 0.46 (95% CI, 0.35-0.61) **(Kovesdy et al., 2008).**

Ravani et al. (2009) found in his study of 168 patients with CKD stages 2-5 that the level of vitamin D decrease as GFR declines, and vitamin D is an independent inverse predictor of disease progression and death in patients with CKD stages 2-5. In addition, nutritional vitamin D supplementation in hemodialysis patients have shown various improvements in intermediate outcomes including increased circulating vitamin D and albumin concentrations as well as decreased calcium, PTH, inflammatory cytokines, left ventricular mass index, and erythropoietin stimulating agent dose **(Matias et al., 2010 and Stubbs et al., 2010).** In this context, small randomized trials have found that treatment with active vitamin D analogs decreases proteinuria and delays progression of kidney disease in patients with mild to moderate CKD **(de Zeeuw et al., 2010).**

The classic biochemical abnormalities in CKD–mineral bone disease are hypocalcemia, hyperphosphatemia, hyperparathyroidism, hypovitaminosis D and elevated fibroblast growth factor-23 **(Cunningham et al., 2011).** In a

cohort study of 1,099 advanced CKD patients, **Kendrick et al. (2012)** found that only 17.2% of subjects had vitamin D concentrations greater than 30 ng/ml, whereas 69.4% had plasma vitamin D concentration ranging between 10-30 ng/ml; the remaining 13.4% were severely vitamin D-deficient (<10 ng/ml). In this regard, **Nigwekar et al. (2012); Rozita et al. (2013) and Kim and Kim. (2014)** reported that vitamin D deficiency or insufficiency is common in patients with CKD, and serum levels of vitamin D appear to have an inverse correlation with kidney disease.

CHAPTER 3
METHODOLOGY

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 **(Mann, 2003 and Song and Chung, 2010).**

3.2 Study population

The study population comprised CKD patients aged 40-65 years attending Kidney unit at Al-Shifa hospital and Nasser Medical Complex in Gaza strip. Control group was apparently healthy persons.

3.3 Sample size and sampling

CKD patients aged 40 to 65 years were taken from Kidney unit at Al-Shifa hospital and Nasser Medical Complex in Gaza Strip. Control healthy individuals were selected from the general population. Cases and controls were matched for age and gender. The sample size calculations were based on the formula for case-control studies. EPI-INFO statistical package version 3.5.1 was used with 95% CI, 80% power and 50% proportion as conservative and OR > 2. The sample size in case of 1:1 ratio of case control was found to be 40:40. For a no-response expectation, the sample size was increased to 42 patients. The controls also consisted of 42 apparently healthy individuals.

3.4 Exclusion criteria

- Cases and controls whose aged under 40 years and above 65 years old.
- Pregnant women.
- Subjects with history of cancer.
- Patients with other chronic diseases such as diabetes .
- Patients who take hormone replacement therapy or corticosteroid therapy.

3.5 Ethical consideration

The necessary approval to conduct the study was obtained from Helsinki committee in the Gaza Strip.

3.6 Data collection

3.6.1 Questionnaire interview

A meeting interview was used for filling in a questionnaire which designated for matching the study need *(Annex 1)*. All interviews were conducted face to face by the researcher herself. During the study the interviewer explained to the participants any of the confused questions that will not clear to them. Most questions were the yes/no question which offers a dichotomous choices and multiple choice **(Backestrom and Hursh-Cesar, 2012).** The validity of the questionnaire was tested by six specialists in the fields of nephrology, epidemiology, public health, biochemistry and nutrition. The questionnaire was piloted with 8 patients not included in the study. The questionnaire included questions on sociodemographic data (Age, education, employment, family income/month and family history of CKD), life style (Smoking, diet, physical activity), and clinical data (Age at diagnosis and duration of CKD).

3.6.2 Body mass index

Body mass index was calculated as the ratio of body weight in Kg/height in square meter. Medical balance (Seca Model 762, Germany) was used for weight measurement. People with BMI=18.5-24.9 were considered to have

normal weight, people with BMI=25.0-29.9 were classified overweight, people with BMI≥30.0 were considered obese **(WHO, 2012).**

3.6.3 Blood collection and processing

Blood samples were collected from 42 CKD patients and 42 controls. Fasting overnight venous blood sample (about 6 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. Then serum samples were obtained by centrifugation at room temperature at 4000 rpm/10 minutes for biochemical analysis.

3.7 Biochemical analysis

3.7.1 Determination of serum vitamin D

25-hydroxy (25-OH) Vitamin D enzyme linked immunoassay (ELISA) is designed by Calbiotech, Inc for the quantitation of total 25-OH Vitamin D in human serum and plasma (**Bikle, 2010).**

Principle

The kit is a solid phase (ELISA), based on the principal of competitive binding. Anti-Vitamin D antibody coated wells are incubated with Vitamin D standards, controls, samples, and Vitamin D-Biotin conjugate at room temperature for 90 minutes. During the incubation, a fixed amount of biotin-labeled vitamin D competes with the endogenous Vitamin D in the sample, standard, or quality control serum for a fixed number of binding sites on the anti-Vitamin D antibody. Following a wash step, bound Vitamin D-Biotin is detected with Streptavidin-HRP (Horseradish peroxidase). Streptavidin-HRP conjugate immunologically bound to the well progressively decreases as the concentration of Vitamin D in the specimen increases. Unbound SA-HRP conjugate is then removed and the wells are washed. Next, a solution of TMB (Tetramethylbenzidine) Reagent is added and incubated at room temperature for 30 minutes, resulting in the development of blue color. The color development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450 nm. A standard curve is obtained

by plotting the concentration of the standard versus the absorbance. The color intensity will be inversely proportional to the amount of 25 (OH)D in the sample. The assay measures both Vitamin D2 and D3. The total assay procedure run time is 2.5 hours.

Composition of reagents

Materials provided 96 Tests	
Microwell plate coated with anti Vitamin D	12x8x1
Vitamin D Standard Set: 7 vials (ready to use)	0.25 ml
Vitamin D Control Set: 2 vials (ready to use)	0.25 ml
Biotinylated 25 (OH)D Reagent: 1 Vial (51X)	0.5 ml
Assay Diluent, 1 bottle	24 ml
Streptavidin-HRP, 1 bottle (ready to use)	23 mL
Stop Solution, 1 bottle (ready to use)	12 mL
TMB Substrate, 2 bottles (ready to use)	2 x 12 ml
Microplate sealing film	2
Wash Concentrate 20X, 1 bottle	25 ml

Preparation of reagent

Before running the test, prepare the following:

1. Standards and Reagents:

Standards are serum-based solutions and stable when stored at -2-8°C, protected from light, until the expiration date on the label. Equilibrate the needed volume of standards and reagents to room temperature before use.

2. 51X Biotin conjugate: Immediately before use, prepare 1X working solution at 1:51 with assay diluent (e.g. Add 0.1ml of the 50x Vitamin D-Biotin conjugate concentrate to 5ml of assay diluent). Remaining Assay Diluent must be stored at 2-8°C in dark and tightly capped.

3. Prepare 1X Wash Buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-24°C).

Analytical procedure

All reagents and specimens must be allowed to come to room temperature before use. All reagents must be GENTLY mixed without foaming. Once the procedure has started, all steps should be completed without interruption.

1. Dispense 10μl of 25(OH)D Standards, controls and samples into each well, as required.

2. Dispense 200µl working solution of biotinylated 25(OH)D reagent, into each well.

3. Carefully mix the contents in the wells for 20 seconds using a plate shaker at 200-400 rpm (or equivalent motion). Remove from shaker and cover the plate with the adhesive plate seal making sure there is a complete seal over each well.

4. Incubation #1 – Incubate sealed plate for 90 minutes at room temperature.

5. Carefully remove the plate seal.

6. Briskly shake out the contents of the wells into a waste reservoir.

7. Wash# 1 - Dispense 300µl of 1X Wash Buffer into each well, and then briskly shake out the 1X Wash Buffer into a waste reservoir. Strike the wells sharply on absorbent paper to remove residual droplets. Repeat 2 more times for a total of 3 washes.

8. Dispense 200µl of enzyme conjugate (Streptavidin-HRP) into each well.

9. Incubation #2 - Incubate for 30 minutes, at room temperature.

10. Briskly shake out the contents of the wells into a waste reservoir.

11. Wash # 2 - Dispense 300µl of 1X Wash Buffer into each well, and then briskly shake out the 1X Wash Buffer into a waste reservoir. Strike the wells sharply on absorbent paper to remove residual droplets. Repeat 2 more times for a total of 3 washes.

12. Using a multi-channel pipette, dispense 200 µl of TMB Substrate into each well.

13. Incubation #3 - Incubate for 30 minutes at room temperature, preferably in the dark.

14. Stop - Dispense 50 µl of Stop Solution into each well to stop the enzymatic reaction. Carefully mix plate contents for 20-30 seconds.

15. Read absorbance on ELISA Reader at 450 nm within 10 minutes of adding the Stop Solution.

Reference values

Deficient	(<10 ng/dl)
Insufficient	(10-30 ng/dl)
Sufficient	(>30 ng/dl)

3.7.2 Determination of serum urea

Serum urea is determined by using colorimetric test **(Fawcett and Scott, 1960)** using DiaSys reagent kits.

Principle

$$\text{Urea} + 2H_2O \xrightarrow{\text{Urease}} 2NH_3 + H_2CO_3$$

$$\text{2-Oxoglutarate} + NH_3 + NADH \xrightarrow{GLDH} \text{L-Glutamate} + NAD^+ + H_2O$$

Reagents

Concentrations are those in the final test mixture.

Reagent	**Concentration**
R1: TRIS	120 mmol/l
2- Oxoglutarate	7 mmol/l
ADP	0.6 mmol/l
Urease	≥ 0.6 ku/l
GLDH	≥ 1 ku/l
R2: NADH	0.25 mmol/l
Standard	50 mg/dl

Assay procedure

The working solution was prepared by mixing 4 parts of R1 with 1 part of R2.

Wavelength: 340 nm

Optical path: 1cm

Temperature: 37 ºC

Measurement: against distilled water.

- Ten microliters of standard (sample or control) was added to 1ml of working reagent and mixed well.
- The mixture was incubated for 30 sec then absorbance (A1) was recorded.
- After exactly further 60 sec the absorbance (A2) was measured.

Calculation

$\Delta A = (A2 - A1)$ sample or standard

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

Reference value

(Palestinian Clinical Laboratory Tests Guide, PCLTG, 2005)

Adult	13 - 43 mg/dl

3.7.3 Determination of serum creatinine

Serum creatinine was determined by using kinetic test without deproteinization according to Newman and Price method **(Newman and Price, 1999)** using DiaSys reagent kits.

Principle

Creatinine forms a colored orange-red complex in an alkaline picrate solution. The difference in absorbance at fixed times during conversion is proportional to the concentration of creatinine in the sample.

Creatinine + Picric acid → creatinine picrate complex

Reagents

Concentrations are those in the final test mixture.

Reagent	Concentration
R1: Sodume hydroxide (pH approx. 13)	0.16 mol/l
R2: Picric acid (pH approx. 1.2)	4.0 mmol/l
Standard	2.0 mg/dl

Assay procedure

The working solution was prepared by mixing 4 parts of R1 with 1 part of R2.

Wavelength: 490 nm

Optical path: 1cm

Temperature: 37 ºC

Measurement: against distilled water.

- Fifty microliters of standard (sample or control) was added to 1ml of working reagent and mixed well.
- The Mixture was incubated for 60 sec then absorbance (A1) was recorded.
- After exactly further 120 sec the absorbance (A2) was measured.

Calculation

ΔA = (A2 – A1) sample or standard

creatinine (mg/dl)=

$$\frac{\Delta A \text{ sample} \times \text{concentration of standard}}{\text{standard}} \Delta A$$

Reference value (in serum) **(PCLTG, 2005)**

Adult: M	0.6 - 1.2 mg/dl
F	0.5 - 1.1 mg/dl

3.7.4 Determination of serum uric acid

Serum uric acid is determined by enzymatic photometric test with TBHBA (2,4,6-tribromo-3-hydroxybenzoic acid) **(Fossati et al., 1980)** using DiaSys reagent kits.

Principle:

Uric acid is oxidized to allantoin by uricase. The generated hydrogen peroxide reacts with 4-aminoantipyrine and 2,4,6-tribromo-3-hydroxybenzoic acid (TBHBA) to quinoneimine.

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

$$\text{TBHBA} + \text{4-aminoantipyrine} + 2H_2O_2 \xrightarrow{\text{POD}} \text{Quinoneimine} + 3\,H_2O$$

Reagents:

Reagent	Components	Concentrations
Reagent 1	Phosphate buffer pH 7.0	100 mmol/l
	TBHBA	1 mmol/l

Reagent 2	Phosphate buffer pH 7.0 4-Aminoantipyrine $K_4[Fe(CN)_6]$ Peroxidase (POD) Uricase	100 mmol/l 0.3 mmol/l 10 µmol/l ≥ 2 kU/L ≥ 30 U/L
Reagent 3	Standard	6.0 mg/dl

Substrate start:

The reagents are ready to use.

Sample start:

Mix 4 part of R1 with 1 part of R2 (e.g. 20 ml R1 + 5 ml R2) = monoreagent. This reagent is stable for 3 months if stored at +2 to +8 ^{0}C, and for 2 weeks if stored at +15 to +25 ^{0}C. Protect the monoreagent from light.

Procedure:

Substrate start

Reagent	Blank	Sample or Standard
Sample or Standard	-	20µ
Dist. Water	20µ	-
Reagent 1	1000µ	1000µ
Mix, incubate 5 min, then add:		
Reagent 2	250µ	250µ
Mix, incubate 30min. at 20 – 25 ^{o}C or 10 min. at 37 ^{o}C. Read the absorbance against the reagent blank within 60 min at wavelength 520 nm.		

Sample start

Reagent	Blank	Sample or Standard
Sample or Standard	-	20µ
Dist. Water	20µ	-
Monoreagent	1000µ	1000µ
Mix, incubate 30min. at 20 – 25 ^{o}C or 10 min. at 37 ^{o}C. Read the absorbance against the reagent blank within 60 min at wavelength 520 nm.		

Calculation:

With standard or calibrator

$$\text{Uric acid [mg/dl]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Std/Cal}} \times \text{Conc. of Std/Cal [mg/dl]}$$

Reference value (in serum)

Adult: M	3.4-7.0 mg/dl
F	2.4-6.0 mg/dl

3.7.5 Determination of serum total protein

Serum total protein was determined by photometric test according to Thomas method **(Thomas, 1998)** using DiaSys reagent kits.

Principle

Protein together with copper ions form a violet blue colour complex in alkaline solution. The absorbance of colour is directly proportional to concentration.

Reagents

Concentrations	Components
80 mmol/L 12.8 mmol/L	**Reagent 1:** Sodium hydroxide Potassium sodium tartrate
100 mmol/L 16 mmol/L 15 mmol/L 6 mmol/L	**Reagent 2:** Sodium hydroxide Potassium sodium tartrate Potassium iodide Copper sulphate
5 g/dl	**Standard**

Mono reagent preparation

Four parts of R1 were mixed with1 part of R2 (e.g. 20 ml R1 + 5 ml R2) =one reagent

Procedure

Sample	Blank	
1000 µl	1000 µl	**Monoreagent**
20 µl	-	**Sample**
-	20 µl	**Dist. Water**

Mix, incubate for 5 min at 25°C and read absorbance against the reagent blank within 60 min at 540 nm.

Calculation

The protein concentration in the sample is calculated using the following general formula:

$$\text{Total protein [g/dL]} = \frac{(\Delta A \text{ sample})}{(\Delta A \text{ standard})} \times \text{Conc. St [g/dl]}$$

Reference value (in serum)

Adult	6.4-8.3 g/dl

3.7.6 Determination of serum albumin

Serum albumin was determined by photometric test according to the method described by Johnson and his colleagues **(Johnson et al., 1999)** using DiaSys reagent kits.

Principle

Serum albumin in the presence of bromocresol green at a slightly acid pH produces a color change of the indicator iron yellow-green to green blue.

Reagents

Concentrations	Components
30 mmol/L 0.26 mmol/L	**Reagent** Citrate buffer pH 4.2 Bromocresol green
5g/dl	**Standard**

Assay procedure

Sample	Blank	
1000 µl	1000 µl	**Reagent**
10 µl	-	**Sample**
-	10 µl	**Dist. Water**

Mix, incubate for approx. 10 min. The absorbance was read against reagent blank within 60 min at 540 – 600 nm.

Calculation

Serum albumin concentration in the sample is calculated using the following general formula:

$$\text{Albumin [g/dL]} = \frac{(\Delta \text{ Asample})}{(\Delta \text{ AStandard})} \text{ X Conc. Std [g/dl]}$$

Reference value (in serum)

Adult	3.5-5.0 g/dl

3.7.7 Determination of serum globulin

Globulin was calculated according the following formula:

Globulin = Total protein – Albumin.

3.7.8 Determination of serum calcium

Serum calcium was determined by photometric test with cresolphthalein complexone **(Thomas, 1998)** using DiaSys reagent kits.

Principle

Cresolphthalein complexone reacts with calcium ions in alkaline medium forming a red-violet color. Interference by magnesium is eliminated by addition of 8-hydroxy-quinoline.

Reagents

Reagent	Components	Concentrations
Reagent 1	Ethanolamine Detergent pH 10.7	600 mmol/L
Reagent 2	2-Cresolphthalein complex 8-Hydroxyquinoline Hydrochloric acid pH 1.1	0.06 mmol/L 7 mmol/L 20 mmol/L

Reagent 3	Standard	10 mg/dL

Preparation and stability of working reagent

Four parts of R1 were mixed with 1 part of R2

Stability: 3 days at 2-8 ^{o}C

Procedure

Wavelength 570 nm, Hg 578 nm (550-590 nm)

Temperature 37°C

Cuvette 1 cm light path

Reading against reagent blank was done

	Blank	**Standard**	**Sample**
Working reagent	1 ml	1 ml	1 ml
Distilled water	20 µl	-	-
Standard	-	20µ l	-
Sample	-	-	20 µl

Mixing and reading the optical density (OD) after 5 minute incubation was done. The final color is stable for at least 15 minutes.

Calculation

$$\frac{\text{OD Sample}}{\text{OD Standard}} \times n = \text{sample calcium concentration (mg/dl)}$$

n = standard calcium concentration

Reference value (in serum)

Adult	8.9-10.1 mg/dl

3.7.9 Determination of serum phosphorus

Serum phosphorus was determined by phosphomolybdate UV end point **(Tiez, 1994)** using Ammonium Molybdate Diagnostic K.

Principle

Determination of inorganic phosphate was made according to the following reaction:

$$\text{Ammonium molybdate} + \text{Sulfuric acid} \xrightarrow{\text{Phosphorus}} \text{Phosphomolybdic complex}$$

Reagents

Reagent	Components	Concentrations
Reagent	Sulfuric acid	210 mmol/L
	Ammonium molybdate	650 umol/L
Standard	Phosphorus	5 mg/dl

Preparation and stability of working reagent

The reagent is ready for use

Procedure:

Wavelength 340 nm

Temperature 37°C

Cuvette 1 cm light path

Reading against reagent blank was done

	Blank	Standard	Sample
Reagent	1 ml	1 ml	1 ml
Distilled water	10 μ	-	-
Standard	-	10 μ	-
Sample	-	-	10 μ

Mixing and reading the optical density (OD) after a 5 minute incubation was done. The final color is stable for at least 1 hour.

Calculation

$$\frac{\text{OD Sample}}{\text{OD Standard}} \times n = \text{sample phosphorus concentration (mg/dl)}$$

n = standard phosphorus concentration

Reference value (in serum)

Adult	2.5-4.5 mg/dl

3.8 Statistical analysis

Data were computer analyzed using SPSS/ PC (Statistical Package for the Social Science Inc. Chicago, Illinois USA, version 18.0) statistical package.

- Simple distribution of the study variables and the cross tabulation were applied.
- Chi-square (χ^2) was used to identify the significance of the relations, associations, and interactions among various variables. Yates's continuity correction test, $\chi^2_{(corrected)}$, was used when not more than 20% of the cells had an expected frequency of less than five and when the expected numbers were small.
- The independent sample t-test procedure was used to compare means of quantitative variables by the separated cases into two qualitative groups such as the relationship between cases and controls vitamin D.
- The one-way ANOVA test was used for analysis of variance.
- Pearson's correlation test was applied.
- The results in all the above mentioned procedures were accepted as statistical significant when the p-value was less than 5% ($p<0.05$).
- Range as minimum and maximum values 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.
- SPSS program version 18.0 was also used for correlation graph plotting between vitamin D and other studied parameters as well as for chart graphs plotting.

Chapter 4

Results

4.1 Personal profile of the study population

Table 4.1 summarizes personal profile of the study population. The study included 42 cases (21 males and 21 females) and 42 controls (21 males and 21 females). Age classification showed that 10 (23.8%) cases and 12 (28.5%) controls were ≤45 years old. Age group 46-55 years comprised 15 (35.7%) cases and 16 (38.1%) controls. Cases and controls aged >55 years old were 17 (40.4%) and 14 (33.3%), respectively. The difference between cases and controls in term of age distribution was not significant (χ^2=0.504, P=0.776). The mean ages of cases and controls were 55.3±8.6 and 54.9±8.2 years old with ranges of 40-65. The independent sample t-test also showed no significant difference between mean ages of cases and controls (t=0.305, P=0.761). Analysis of the educational status of the study population showed that 7 (16.6%) cases and 6 (14.3%) controls had a university degree, 3 (7.14%) cases and 6 (14.3%) controls had finished secondary school, 8 (19.0%) cases and 12 (28.5%) controls had finished preparatory school, 9 (21.4%) cases and 11 (26.2%) controls had passed primary school, and 15 (35.7%) cases and 7 (16.6%) controls were illiterate. The difference between various educational levels of cases and controls was not significant ($\chi^2_{(corrected)}$=3.172, P=0.529).

Table 4.1 Personal profile of the study population

Personal profile	Cases (n=42)		Controls (n=42)		Test		p-value
	No.	%	No.	%			
Age (Year)							
≤45	10	23.8	12	28.5	χ^2	0.504	0.776
46-55	15	35.7	16	38.1			
>55	17	40.4	14	33.3			
Mean±SD	55.3±8.6		54.9±8.2		T	0.305	0.761
Range *(min-max)*	*40-65*		*40-65*				
Gender							
Male	21	50.0	21	50.0	χ^2	0.000	1.000
Female	21	50.0	21	50.0			
Education							
University	7	16.6	6	14.3	χ^2	3.172	0.529*
Secondary school	3	7.14	6	14.3			
Preparatory school	8	19.0	12	28.5			
Primary	9	21.4	11	26.2			
Illiterate	15	35.7	7	16.6			

*P-value of $\chi^2_{(corrected)}$ test.
P>0.05: not significant.

4.2 Socioeconomic data of the study population

Socioeconomic data of the study population is provided in Table 4.2. The employed cases and controls were 12 (28.5%) and 22 (52.3%) whereas 30 (71.4%) cases and 20 (47.6%) controls were unemployed. The difference between the two groups was significant (χ^2=4.941, P=0.026). Regarding family income\month, significant difference was also recorded between cases and controls (χ^2=7.939, P=0.019) with CKD more frequent among families with low income. Family history of CKD revealed that 11 (26.1%) cases and 3 (7.14%) controls reported that they had family history of CKD whereas 31 (73.8%) cases and 39 (42.8%) controls had not ($\chi^2_{(corrected)}$=4.200, P=0.040) indicating that family history is associated with CKD. In addition, 8 (19.0%) cases were smokers compared to 5 (11.9%) controls (χ^2=0.819, P=0.365).

Table 4.2 Socioeconomic data of the study population

Socioeconomic data	Cases (n=42)		Controls (n=42)		$\chi 2$	p-value
	No.	%	No.	%		
Employment						
Yes	12	28.5	22	52.3	4.941	0.026
No	30	71.4	20	47.6		
Family income/month (NIS)*						
<1000	23	54.7	12	28.5	7.939	0.019
1000-2000	8	19.0	19	45.2		
>2000	11	26.1	11	26.1		
Family history of CKD						
Yes	11	26.1	3	7.1	4.200	0.040*
No	31	73.8	39	42.8		
Smoking						
Yes	8	19.0	5	11.9	0.819	0.365
No	34	80.9	37	88.0		

*NIS: new Israeli Shekels. *P-value of χ^2 (corrected) test.
P<0.05:Significant, P>0.05:not significant

4.3 Duration of chronic kidney disease and its distribution among patients

The mean duration of CKD among patients was 9.3±10.4 years. The distribution of CKD among patients by the duration of the disease is demonstrated in Table 4.3. Patients with CKD 1-10 years were 29 (69.0%), whereas those with CKD duration of 11-20 years were 7 (16.6%). The rest of patients 6 (14.2%) had CKD for more than 21 years.

Table 4.3 Distribution of CKD among patients (n=42) by the duration of the disease.

Duration of CKD (Year)	No.	%
1-10	29	69.0
11-20	7	16.6
21-34	6	14.2
Mean±SD=9.3±10.4		

4.4 Body mass index of the study population

Table 4.4 shows BMI of the study population. The mean weight of cases compared to controls were 75.1±15.1 *versus* 77.8±16.2 kg, % difference= -3.53, t=0.795 and P=0.429. The mean height of cases compared to controls were 1.65±0.1 *versus* 1.68±0.08 meter, % difference=-1.80, t=1.452 and P=0.150. Therefore, BMI of cases and controls were 27.6±5.2 and 27.4±4.1 kg/m^2, respectively, % difference=0.73 and t=0.164 and P=0.870.

Table 4.4 Body mass index (BMI) of the study population.

Anthropometric measurement	Cases (n=42) mean± SD	Controls (n=42) mean± SD	% difference	T	P-value
Weight (kg)* (min-max)	75.1±15.1 (44-120)	77.8±16.2 *(45-110)*	-3.53	0.795	0.429
Height (m)** *(min-max)*	1.65±0.1 *(1-1.9)*	1.68±0.08 *(1.45-1.9)*	-1.80	1.452	0.150
BMI***	27.6±5.2 *(17.4-45.0)*	27.4±4.1 *(17.7-38.3)*	0.73	0.164	0.870

*Kg: kilogram, **m: meter. ***BMI (Kg/m^2): People with BMI=18.5-24.9 were considered to have normal weight, people with BMI=25.0-29.9 were classified overweight, people with BM≥30.0 were considered obese **(WHO, 2012).** P>0.05: not significant.

4.5 Serum vitamin D level of the study population

Table 4.5 and Figure 4.1 show the mean level of serum vitamin D of the study population. There was a significant decrease in the mean level of vitamin D in cases compared to controls (29.7±12.9 *versus* 35.2±9.9 ng/dl, % difference= -16.9, t=2.171 and P=0.033).

Table 4.5 Serum vitamin D levels of the study population.

Parameter	Case (n=42) mean±SD	Control (n=42) mean±SD	% difference	T	P-value
Vitamin D (ng/dl) *(min-max)*	29.7±12.9 *(7.0-51.0)*	35.2±9.9 *(9.0-53.7)*	-16.9	2.171	0.033

P<0.05: Significant.

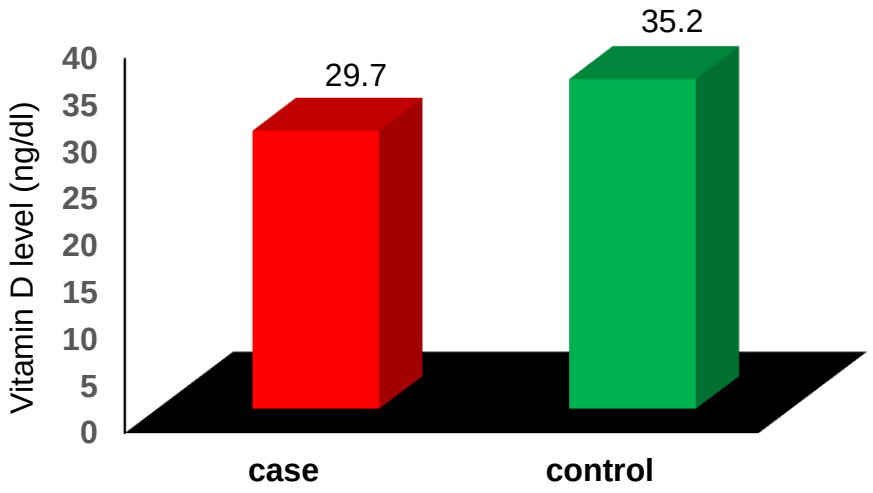


Figure 4.1 Serum vitamin D levels of cases (n=42) and controls (n=42)

4.6 Categories of serum vitamin D levels of the study population

As illustrated in Table 4.6 and Figure 4.2, serum vitamin D level of the study population were classified into 3 different categories: deficient (<10 ng/dl), insufficient (10-30 ng/dl) and sufficient (>30 ng/dl). The number of cases having vitamin D deficient, insufficient and sufficient were 6 (14.3%), 17 (40.5%) and 19 (45.2%), respectively compared to controls of 1 (2.4%),10 (23.8%) and 31 (73.8%), respectively with $\chi^2_{(corrected)}$=6.039 and P=0.042.

Table 4.6 Different categories of serum vitamin D levels of the study population.

Vitamin D	Case (n=42)		Control (n=42)		χ^2	P-value*
	No.	%	No.	%		
Deficient (<10 ng/dl)	6	14.3	1	2.4	6.039	0.042*
Insufficient (10-30 ng/dl)	17	40.5	10	23.8		
Sufficient (>30 ng/dl)	19	45.2	31	73.8		

*P-value of $\chi^2_{(corrected)}$ test, P<0.05: Significant.

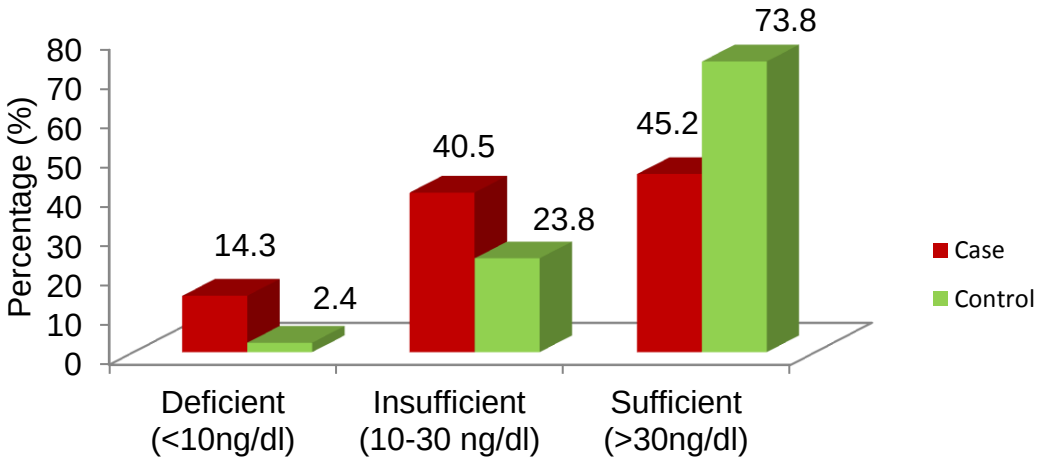


Figure 4.2: Different categories of serum vitamin D levels of cases (n=42) and controls (n=42).

4.7 Kidney function of the study population

The concentration of serum urea, creatinine and uric acid as an indicator of kidney function are illustrated in Table 4.7. Urea, creatinine and uric acid concentrations were significantly higher in cases than in controls (84.6±47.4 and 1.90±1.20 and 7.92±2.29 *versus* 35.7± 13.5 and 0.81±0.27 and 5.18±2.31 mg/dl, % differences 81.3, 80.4 and 41.8, P=0.000, respectively).

Table 4.7 kidney function of the study population.

Parameter (mg/dl)	case (n=42) Mean ±SD	control(n=42) Mean ±SD	% Difference	T	P-value
Urea *(min-max)*	84.6±47.4 *(12-204)*	35.7±13.5 *(13-82)*	81.3	6.428	0.000
Creatinine *(min-max)*	1.90±1.20 *(0.64-6.78)*	0.81±0.27 *(0.43-1.2)*	80.4	5.785	0.000
Uric acid (min-max)	7.92±2.29 (3.6-12.9)	5.18±2.31 (1.20-11.3)	41.8	5.428	0.000

P<0.05: Significant.

4.8 Glomerular filtration rate of the study population

Table 4.8 shows GFR of the study population**.** The mean GFR was significantly decreased in cases compared to controls (62.4±32.5 *versus 124.6±45.4* ml/min/1.73m^2), recording % difference of -66.5, t=7.114 and P=0.000.

Table 4.8 GFR of the study population

Parameter	Case (n=42) mean±SD	Control (n=42) mean±SD	% difference	T	P-value
GFR *Range (min-max)*	62.4±32.5 (14-119)	124.6±45.4 (85-197)	-66.5	7.114	0.000

GFR was calculated from Schwartz equation (NKF, 2002). GFR (ml/min/1.73m²) = 0.55 × length ⁄Scr. P<0.05: Significant.

4.9 Serum protein profile of the study population

Table 4.9 shows serum total protein, albumin and globulin concentrations of the study population**.** The mean concentrations of total protein and albumin were significantly decreased in cases compared to controls (7.0±0.5 and 5.2±0.40 *versus* 7.3±0.6 and 5.4±0.59 mg/dl), recording % difference of -4.2 and -3.8, t=2.928 and t=2.316 and P=0.005 and P=0.023, respectively. Similarly, serum globulin was decreased in cases compared to controls but this change was not significant (1.82±0.6 *versus* 1.92±0.6, % difference=-5.3, t=0.728, P=0.469).

Table 4.9 Serum total protein, albumin and globulin concentrations of the study population

Parameter (mg/dl)	Case (n=42) mean±SD	Control (n=42) mean±SD	% difference	T	P-value
Total protein	7.0±0.5	7.3±0.6	-4.2	2.928	0.005
(min-max)	(6.2-8.7)	(6.5-10.4)			
Albumin	5.2±0.40	5.4±0.59	-3.8	2.316	0.023
(min-max)	(3.6-5.9)	(4.1-8.1)			
Globulin	1.82±0.6	1.92±0.6	-5.3	0.728	0.469
(min-max)	(1.0-3.1)	(1.0-3.6)			

P<0.05: Significant, P>0.05: not significant.

4.10 Serum calcium and phosphorus of the study population

Table 4.10 shows serum calcium and phosphorus concentrations of the study population**.** The mean concentration of serum calcium was significantly decreased in cases compared to controls (8.61±0.77 *versus* 9.12±0.69 mg/dl), recording % difference of -5.8, t=3.221 and P=0.003. On the other hand, serum phosphorus concentration exhibited no significant difference between cases and controls (4.72±0.94 *versus* 4.49±0.85, % difference=4.9, t=1.186 and P=0.239).

Table 4.10 Serum calcium and phosphorus concentrations of the study population

Electrolyte (mg/dl)	Case (n=42) mean±SD	Control (n=42) mean±SD	% difference	T	P-value
Calcium	8.61±0.77	9.12±0.69	-5.8	3.221	0.003
(min-max)	(7.0-10.5)	(7.9-10.8)			
Phosphorus	4.72±0.94	4.49±0.85	4.9	1.186	0.239
(min-max)	(3.4-6.7)	(2.5-6.4)			

P<0.05: Significant, P>0.05: not significant.

4.11 Relations of vitamin D

4.11.1 Serum vitamin D level in relation to sociodemographic data of the study population

Table 4.11 shows the relationship between sociodemographic data and serum vitamin D level of the study population. Serum vitamin D level was significantly lower in individuals who reported family history of CKD than who did not (26.1±13.6 *versus* 33.2±11.5, t=2.167, P=0.038), implying that vitamin D level is associated with family history of the disease. On the other hand, there were no associations between vitamin D and employment or family income.

Table 4.11 Serum vitamin D level in relation to sociodemographic data of the study population

Sociodemographic data	Vitamin D Mean±SD	Statistical test		P-value
Employment				
Yes	36.7±14.3	t	1.349	0.181
No	31.8±11.2			
Family income				
<1000	29.3±11.2	F	2.296	0.107
1000-2000	33.8±9.50			
>2000	35.7±13.8			
Family history				
Yes	26.1±13.6	t	2.167	0.038
No	33.2±11.5			

P<0.05: Significant and P>0.05: Not significant.

4.11.2 Vitamin D level in relation to CKD duration among the study population

Table 4.12 shows a progressive decrease in serum vitamin D level with increasing the duration of CKD. However, this inverse relationship was not significant (F=2.048, P=0.143).

Table 4.12 Serum vitamin D level in relation to CKD duration among the study population

Duration of CKD (Year)	No.	Vitamin D (ng/dl) mean±SD	F	P-value
1-10	29	37.0±12.8		
11-20	7	34.7±11.3	2.048	0.143
21-34	6	26.4±13.2		

P>0.05: Not significant.

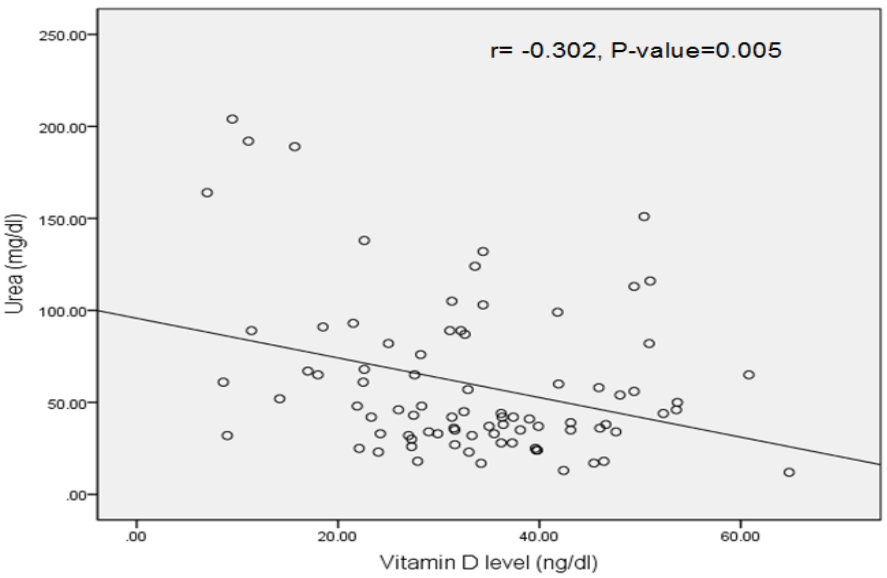
4.11.3 Vitamin D levels in relation to urea, creatinine and uric acid of the study population

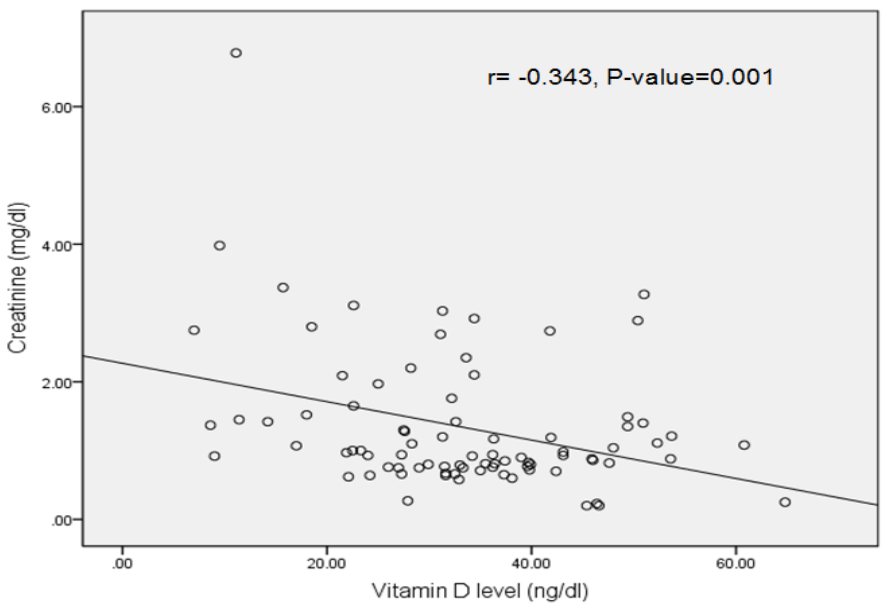
Vitamin D levels in relation to urea, creatinine and uric acid of the study population is provided in Table 4.13 and Figure 4.3. Vitamin D showed negative significant correlations with urea, creatinine and uric acid levels (r=-0.302, r= -0.343 and r=-0.249; P=0.005, P= 0.001 and P=0.022, respectively).

Table 4.13 Serum vitamin D levels in relation to urea, creatinine and uric acid of the study population

Parameter (mg/dl)	Vitamin D (ng/dl) Pearson correlation (r)	P-value
Urea	-0.302	0.005
Creatinine	-0.343	0.001
Uric acid	-0.249	0.022

P<0.05: Significant.


r= -0.302, P-value=0.005
Urea (mg/dl)
250.00
200.00
150.00
100.00
50.00
.00
.00
20.00
40.00
60.00
Vitamin D level (ng/dl)


r= -0.343, P-value=0.001
Creatinine (mg/dl)
6.00
4.00
2.00
.00
.00
20.00
40.00
60.00
Vitamin D level (ng/dl)

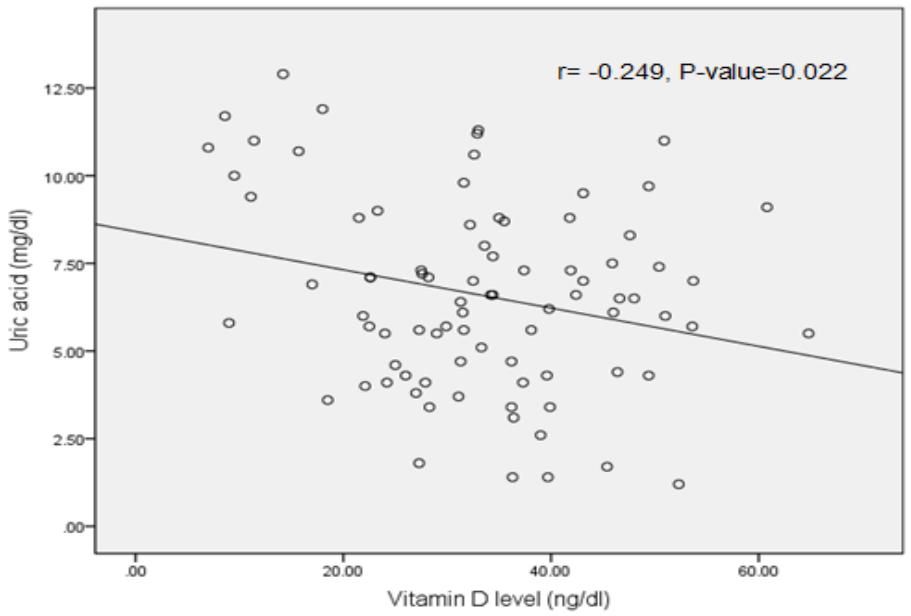


Figure: 4.3. Serum vitamin D levels in relation to urea, creatinine and uric acid of the study population.

4.11.4 Vitamin D level in relation to GFR of the study population

Vitamin D level in relation to GFR of the study population is provided in Table 4.14 and Figure 4.4. Vitamin D showed a positive significant correlation with GFR level (r=0.258, P=0.020).

Table 4.14 Serum vitamin D level in relation to GFR of the study population

Parameter	Vitamin D (ng/dl)	
	Pearson correlation (r)	P-value
GFR	0.258	0.020

P<0.05: Significant.

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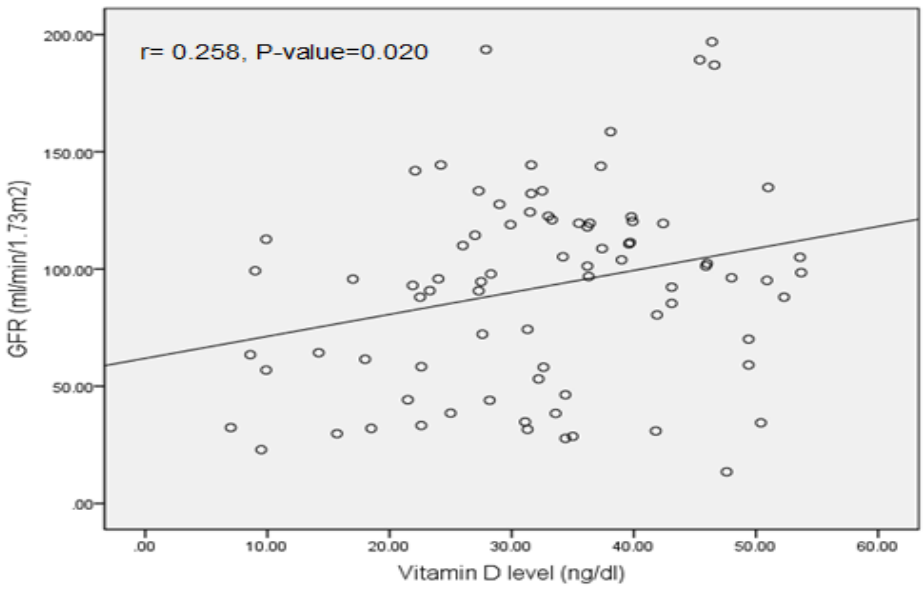


Figure: 4.4. Serum vitamin D level in relation to glomerular filtration rate (GFR) of the study population.

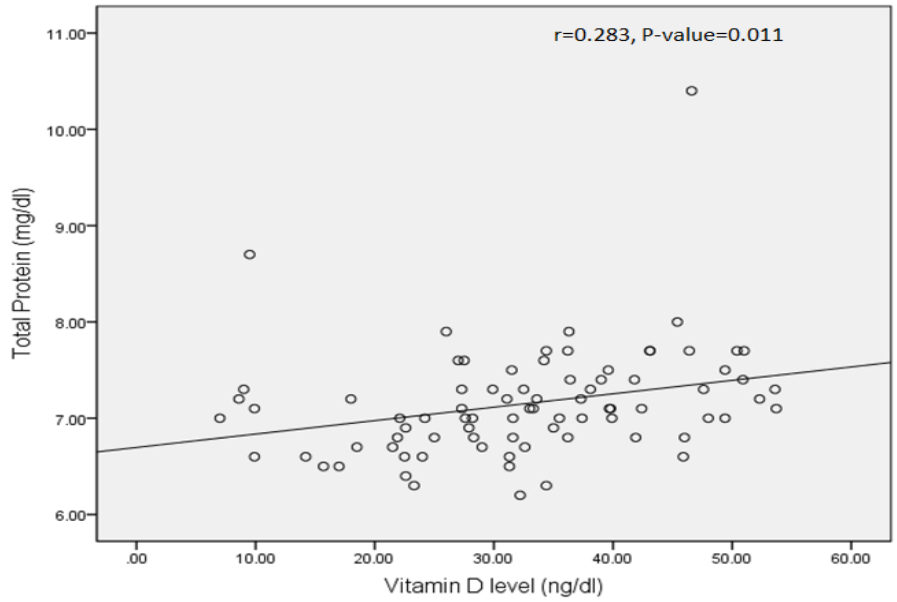
4.11.5 Vitamin D levels in relation to protein profile of the study population

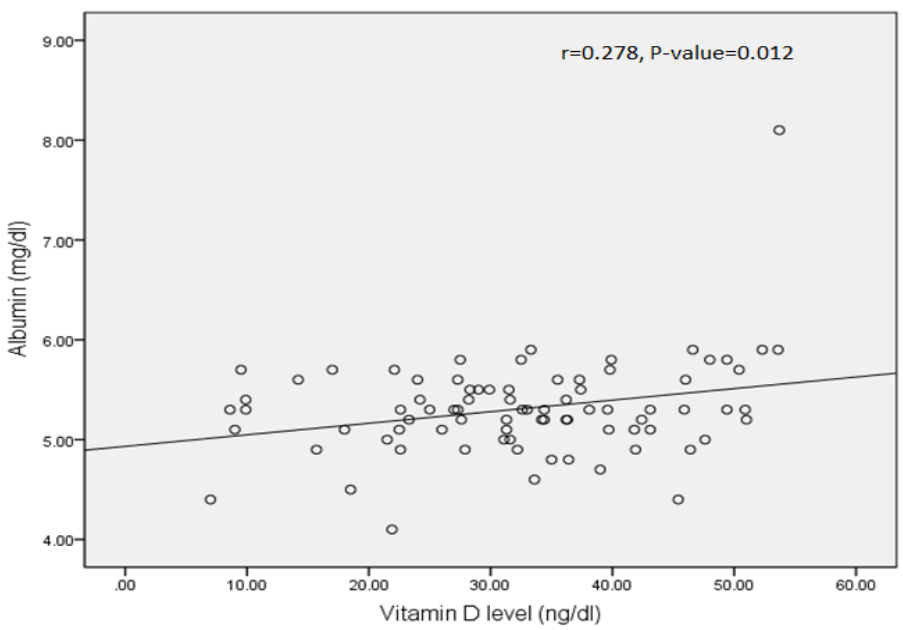
Vitamin D levels in relation to total protein, albumin and globulin of the study population is illustrated in Table 4.15 and Figure 4.5. Vitamin D showed positive significant correlations with total protein and albumin levels (r=0.283, P=0.011 and r=0.278, P=0.012, respectively). However, a positive none significant correlation was recorded between vitamin D and globulin level (r=0.159, P=0.156).

Table 4.15 Serum vitamin D levels in relation to total protein, albumin and globulin of the study population

Parameter (mg/dl)	Vitamin D (ng/dl) Pearson correlation (r)	P-value
Total protein	0.283	0.011
Albumin	0.278	0.012
Globulin	0.159	0.156

P<0.05: Significant, P>0.05: not significant.


r=0.283, P-value=0.011
Total Protein (mg/dl)
11.00
10.00
9.00
8.00
7.00
6.00
.00
10.00
20.00
30.00
40.00
50.00
60.00
Vitamin D level (ng/dl)


r=0.278, P-value=0.012
Albumin (mg/dl)
9.00
8.00
7.00
6.00
5.00
4.00
.00
10.00
20.00
30.00
40.00
50.00
60.00
Vitamin D level (ng/dl)

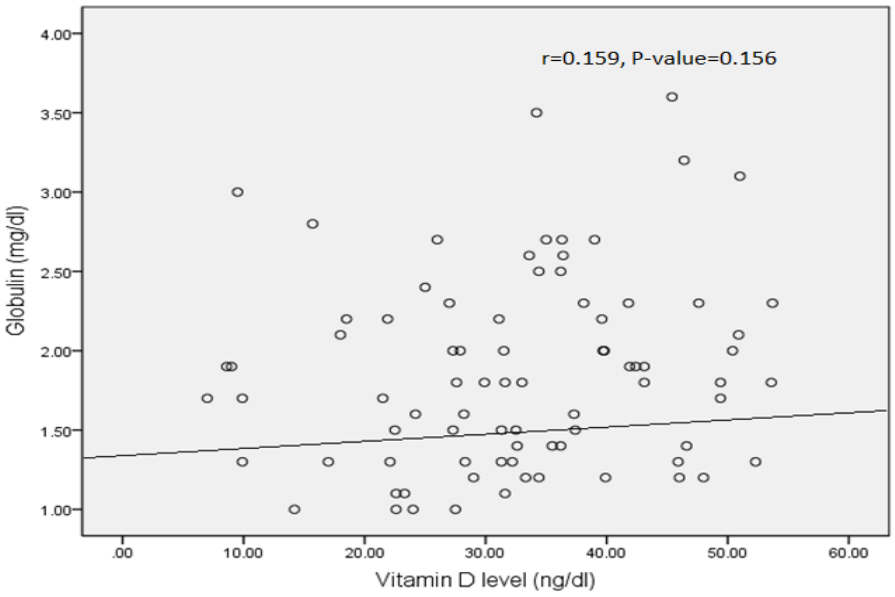


Figure: 4.5. Serum vitamin D levels in relation to total protein, albumin and globulin concentrations of the study population.

4.11.6 Vitamin D levels in relation to calcium and phosphorus of the study population

Vitamin D levels in relation to calcium and phosphorus of the study population is presented in Table 4.16 and Figure 4.6. Vitamin D showed a positive significant correlation with calcium level (r=0.562, P=0.001). However, a negative none significant correlation was recorded between vitamin D and phosphorus level (r=-0.168, P=0.125).

Table 4.16 Serum vitamin D levels in relation to calcium and phosphorus of the study population

	Vitamin D (ng/dl)	
Electrolyte (mg/dl)	**Pearson correlation (r)**	**P-value**
Calcium	0.562	0.001
Phosphorus	-0.168	0.125

P<0.05: Significant, P>0.05: not significant.

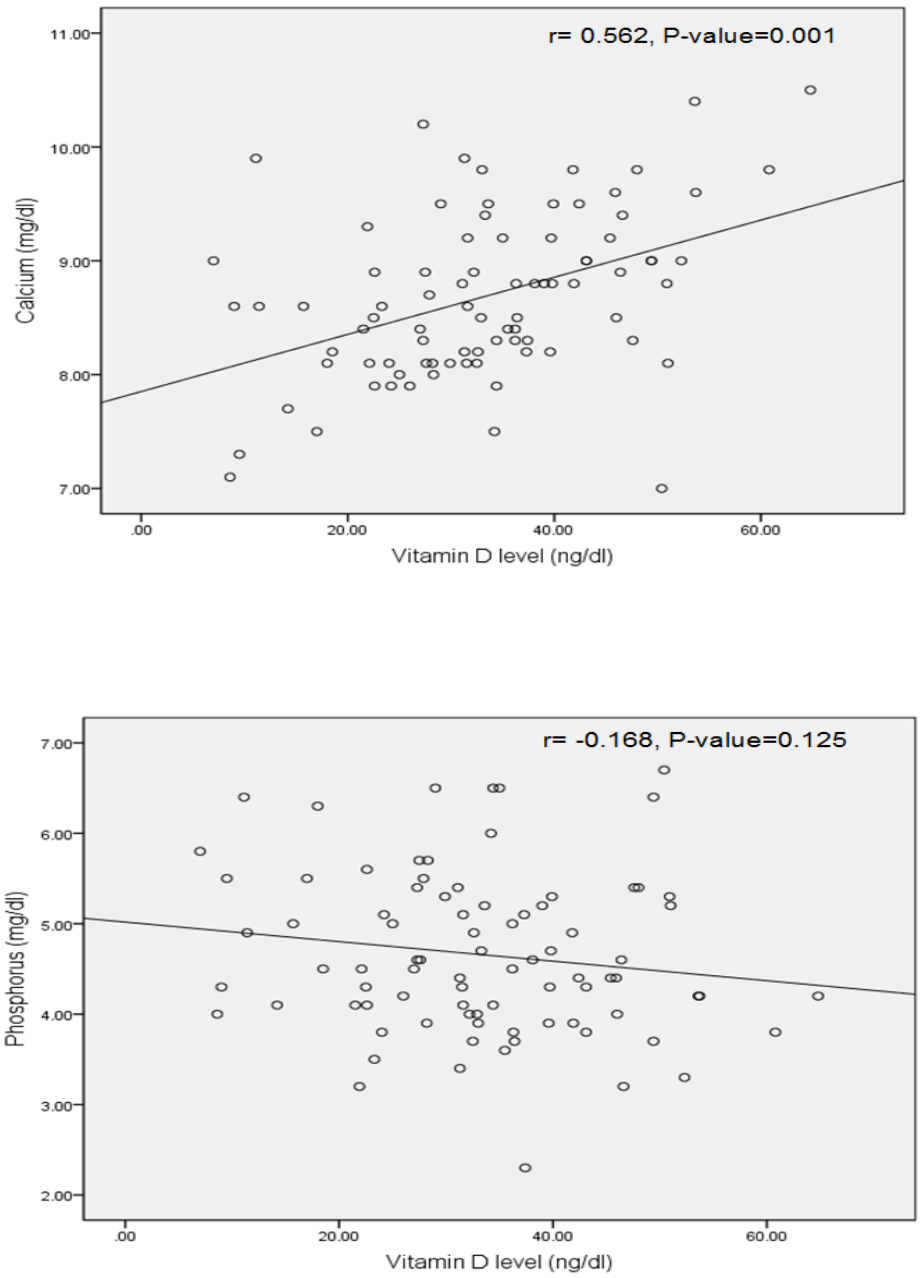


Figure: 4.6. Serum vitamin D levels in relation to serum calcium and phosphorus concentrations of the study population.

Chapter 5

Discussion

Chronic kidney disease is a serious health problem globally as well as in Palestine. The prevalence of CKD among adults was estimated at 7.7-13% worldwide **(Gouda et al., 2011; Nakai et al., 2013 and CDC, 2014).** In most cases, CKD patients end with renal failure **(U.S. Renal Data System, USRDS, 2009)**. The total prevalence of ESRD among Palestinian patients was 240.3 people per million population **(Khader et al., 2013).** Despite that, the published data on the disease were few in the Gaza strip and most of information emerged as annual reports produced by the Palestinian Ministry of Health. The available biochemical tests of CKD were limited to routine traditional kidney function including urea and creatinine tests when the patient visited the clinic. This necessitated further assessment of other biochemical features in blood such as vitamin D, its deficiency was recently linked to CKD **(Cunningham et al., 2011; Satirapoj et al., 2013 and Obi et al., 2015).** Therefore, supplementation of vitamin D to CKD patients in the Gaza hospitals and clinics may be useful in the disease management.

5.1 Sociodemoghraphic data of the study population

The present study is a case-control investigation included 42 CKD patients (21 males and 21 females) with mean age 55.3±8.6 years and 42 controls (21 males and 21 females) with mean age 54.9±8.2 years. Chronic kidney disease was more prevalent among unemployed cases compared to their counterparts of controls. Similar results were previously documented by **White et al. (2008) and Abu Nada, (2012)** who showed that unemployment increased the incidence of CKD. Regarding family income, CKD was more prevalent among families with less income/month. This result is in agreement with that obtained by **NKF, (2002) and Bello et al. (2008)** who pointed out that low income was associated with greater impairments' in functioning and well-being in patients with CKD. In addition, **Zhang et al. (2012)** reported that the prevalence of CKD varied greatly between geographical regions and this might be related to variability in lifestyles and economic development. Furthermore, family history of CKD was reported by higher number of cases

than controls, indicating that family history is associated with CKD. Such finding is in accordance with other studies **(Johnson, 2012 and CDC, 2014).**

5.2 Duration of chronic kidney disease and its distribution among patients

Data presented in this study showed that more than two thirds of cases had CKD since 10 years or less. This confirmed the idea that CKD has long asymptomatic preclinical phase which frequently goes undetected, particularly in developing world where CKD is prevalent and most of people did not had routine medical examination **(Nigwekar et al., 2014).**

5.3 Serum vitamin D levels of the study population

The present results revealed that the number of cases having vitamin D deficiency and insufficiency were significantly higher than that of controls. Vitamin D deficiency and insufficiency were found to be prevalent among CKD patients **(Kendrick et al., 2012 and Satirapoj et al., 2013).** When compared with controls, cases showed significant decrease in the mean level of serum vitamin D. This means that deficiency in vitamin D levels are linked to CKD. In consistent with this result, the prevalence of vitamin D deficiency has been reported in CKD patients **(Holick, 2007; de Boer et al., 2011 and Obi et al., 2015).** In addition, **Pilz et al. (2011)** concluded that supplementation of vitamin D is associated with a lower risk of CKD. The kidney plays a central role in vitamin D metabolism and regulation of its circulating levels (**Kannan and Lim, 2014 and Obi et al., 2015).** Therefore, impaired renal function may lead to vitamin D deficiency, as has been found in patients with CKD.

Several mechanisms involved in decreased production of vitamin D that occur in CKD were proposed:

1. Decrease in GFR may limit the delivery of vitamin D substrate 25(OH)D to 1α-hydroxylase enzyme, resulting in reduced production of vitamin D **(Dusso et al., 2011).** The significant decrease in GFR recorded in the present study do coincides with this view.
2. Downregulation of renal 1α-hydroxylase may be attributed to a) hyperphosphatemia resulting from persistent phosphate retention, b)

increased fibroblast growth factor-23 due to declining kidney function, c) acidosis, hyperuricemia and uremia **(Gutierrez et al., 2005; Usatii et al., 2007 and Kuro, 2011).**

3. A decrease in renal mass as reported in CKD may limit the amount of 1α-hydroxylase enzyme available for the production of vitamin D **(Andress, 2006).**

4. Alterations in cytochrome P 450 enzymes (CYP enzymes) abundance **(Bosworth and de Boer, 2013).**

When related to family history, vitamin D levels were found to be significantly lower in individuals who reported family history of CKD than who did not. This indicates that CKD patients with family history of the disease are more likely to develop vitamin D deficiency. In this instance, vitamin D supplementation to such patients could be useful. Its accepted that vitamin D treatment improves CKD **(Pilz et al., 2011 and Kim and Kim, 2014).** Concerning CKD duration, the present results revealed that vitamin D level was inversely related to CKD duration. **Ravani et al. (2009)** showed that serum vitamin D is an inverse predictor of kidney disease progression.

5.4 Kidney function of the study population

Urea, creatinine and uric acid were significantly increased in cases compared to controls whereas GFR was significantly decreased. Similar results were documented **(Chen et al., 2009; Renal Resource Center, 2010 and Abu Nada, 2012).** Accumulation of urea, creatinine and uric acid in the blood is a predictable consequence of progressive decline of GFR as CKD proceeded. Pearsons correlation test showed a significant negative correlation of vitamin D with urea, creatinine and uric acid, whereas strong positive correlation was recorded with GFR. Similar results were reported by **kim and kim (2014).** This implies that vitamin D deficiency is linked to kidney function. In addition, several observational studies have demonstrated an important link between vitamin D deficiency, impaired GFR, and increased mortality in patients with CKD **(Mehrotra et al., 2009; Pilz et al., 2011 and Urena-Torres et al., 2011).**

5.5 Serum protein profile of the study population

As illustrated in the present study, there was a significant decrease in the mean level of total protein and albumin in cases compared to controls. Similarly serum globulin was decreased without significance. These results are in agreement with that found by **Levey (2002), and Abu Nada, (2012)**. It is known that, in CKD, there is a loss of serum protein and albumin in urine leading to proteinurea **(Wen et al., 2008; Guh, 2010 and Campbell and Weir, 2015)**. In addition to its loss in urine, serum protein may breakdown to form urea that further contributing to elevation of serum urea concentration noted in CKD patients. When related to vitamin D levels, total protein and albumin showed significant positive correlations indicating that these parameters are interchangeable in CKD. However none significant positive correlation was found with globulin. Several studies have shown a correlation between vitamin D deficiency and an increased degree of albuminuria **(de Boer et al., 2007 and Isakova et al., 2011).**

5.6 Serum calcium and phosphorus of the study population

The mean concentration of serum calcium was significantly decreased in cases compared to controls whereas serum phosphorus concentration exhibited no significant increase between cases and controls. In this context, one can say that phosphate retention in the kidney can lead to a decrease in the serum calcium level. Hypocalcemia was reported in CKD (**Wolf et al., 2007 and Kalantar-Zadeh, 2008).** Impairment in renal reabsorption of calcium could be stand behind hypocalcemia observed in CKD. When related to vitamin D level, serum calcium showed a positive correlation, whereas serum phosphorus displayed a negative correlation with vitamin D. These results are in agreement with that demonstrated by **Sai et al. (2011) and Kendrick et al. (2012).** Hypocalcemia accompanied with vitamin D deficiency may be attributed to the fact that vitamin D enhances intestinal calcium absorption (**Christakos et al., 2011 and Takahashi et al., 2014).**

Chapter 6

Conclusions and Recommendations

6.1 Conclusions

1. Chronic kidney disease was more prevalent among unemployed individuals, families with low income and individuals with family history of the disease.
2. More than two-thirds of cases had CKD since 10 years or less.
3. The mean level of serum vitamin D was significantly lower in cases compared to controls. The number of cases having vitamin D deficiency and insufficiency were significantly higher than that of controls.
4. Urea, creatinine and uric acid concentrations showed significant increase in cases compared to controls, whereas GFR was significantly decreased in cases compared to controls.
5. The mean levels of total protein and albumin were significantly lower in cases compared to controls.
6. Serum calcium was significantly lower in cases compared to controls whereas, serum phosphorus showed none significant increase in cases.
7. Serum vitamin D was found to be significantly lower in individuals with family history of CKD.
8. Serum vitamin D showed significant negative correlations with urea, creatinine and uric acid and significant positive correlation with GFR.
9. Serum vitamin D was positively correlated with total protein, albumin and globulin.
10. Serum vitamin D exhibited significant positive correlation with calcium and none significant negative correlation with phosphorus.

6.2 Recommendations

1. Introducing of vitamin D test for CKD patients in Gaza hospitals is highly recommended.
2. Frequent monitoring of vitamin D levels particularly in CKD patients especially those with family history of the disease.
3. Supplementation of vitamin D to CKD patients could be useful in management and treatment strategy of the disease .

Annex 1

Questionnaire

I. Sociodemographic data

1. Code number ……

2. Name (optional) ……

3. Age in years …….

4. **Sex** a. Male b. Female

5. Education.

a. illiterate b. primary school c. preperatary school d. secondary school e. University

6. Employment a. Yes b. No

7. Family income per month (shekel)

a. Less than 1000 b. 1000-2000 c. More than 2000

8. Family history of chronic kidney disease ?

a. Yes b. No

II.life style

9. Do you follow any particular diet?

a. Yes b. No

10. Do you do physical activity?

a. Yes b. No

III. clinical data

11. Duration of CKD?

CHAPTER 7

REFERENCES

Abu annai M. and O'Keefe J.H. (2011): Vitamin D and cardiovascular health. Primary Care Cardiovascular Journal 4: 59-62.

Abu Nada A.A. (2012): Homocysteine levels in chronic kidney disease patients from Gaza Governorate, Gaza Strip. Master thesis, The Islamic University of Gaza, Gaza Strip, Palestine.

Adams J.S. and Hewison M. (2010): Update in vitamin D. The Journal of Clinical Endocrinology and Metabolism 95: 471-478.

Afsaneh Talaei, Mohnaz Mohamadi and Zahra Adgi (2013): The effect of Vitamin D on Resistance in Patients with Type 2 Diabetes. Journal of Diabetology and Metabolic Syndrome 5:8.

Alsuwaida A.O., Farag Y.M., Al Sayyari A.A., Mousa D., Alhejaili F., Al-Harbi A., Housawi A., Mittal B.V. and Singh A.K. (2010): Epidemiology of chronic kidney disease in the Kingdom of Saudi Arabia (SEEK-Saudi investigators) - a pilot study. Saudi Journal Kidney Disease and Transplantation 21 (6): 1066-1072.

Anderson J.L., Adams C.D., Antman E.M., Bridges C.R., Califf R.M., Casey D.E. and Chavey W.E. (2007): Guidelines for the management of patients with unstable angina/non–ST-elevation myocardial infarction. Journal of American College of Cardiology 50: 150-157.

Andress D.L. (2006): Vitamin D in chronic kidney disease: a systemic role for selective vitamin D receptor activation. Kidney International 69: 33-43.

Antico A., Tampoia M., Tozzoli R. and Bizzaro N. (2012): Can supplementation with vitamin D reduce the risk or modify the course of autoimmune diseases? A systematic review of the literature. Autoimmunity Reviews 12: 127-136.

Backestrom C., and Hursh-Cesar G. (2012): Survey research, Pennsylvania, United States: Literary Licensing, LLC, 2012.

Balch P.A. (2001): Prescription for nutritional healing: The A-To-Z Guide to Supplements. 3ed. Page45. AVERY, New york.

Barrett E., Barman M., Boitano S. and Brooks L. (2010): Ganong's review of medical physiology, 23rd. McGraw Hill, Boston.

Barsoum R.S. (2006): Chronic kidney disease in the developing world. The New England Journal of Medicine (354): 997-999.

Bauer C., Melamed M.L. and Hostetter T.H. (2008): Staging of chronic Kidney disease: Time for a course correction. Journal of the American Society of Nephrology 19: 844-846.

Baumgarten M. and Gehr T. (2011): Chronic kidney disease: detection and evaluation. American Family Physician 84(10): 1138-1148.

Bello A.K., Peters J. and Rigby J. (2008): Socioeconomic status and chronic kidney disease at presentation to a renal service in the United Kingdom. Clinical Journal of the American Society of Nephrology 3: 1316-1323.

Bikle D.D. (2010): Vitamin D and the skin. Journal of Bone and Mineral Metabolism 28: 117-130.

Bosworth C. and de Boer I.H. (2013): Impaired vitamin D metabolism in CKD. Seminars in Nephrology 33(2): 158-168.

Bosworth C., de Boer I.H., Targher G., Kendrick J., Smits G. and Chonchol M. (2012): The effect of combined calcium and cholecalciferol supplementation on bone mineral density in elderly women with moderate chronic kidney disease. Clinical Nephrology 77(5): 358-365.

Campbell D. and Weir M.R. (2015): Defining, treating, and understanding chronic kidney disease—A Complex Disorder. The Journal of Clinical Hypertension p (1-14).

Centers for Disease Control and Prevention (CDC), (2014): National Chronic Kidney Disease Fact Sheet, 2014.

Chagas C., Borges M., Martini L. and Rogero M. (2012): Focus on vitamin D, inflammation and type 2 diabetes. Nutrients 4: 52-67.

Chen J., Gu D. and Chen C.S. (2007): Association between the metabolic syndrome and chronic kidney disease in Chinese adults. Nephrology Dialysis Transplantation (22): 1100-1106.

Chen Y.C., Su C.T., Wang S.T., Lee H.D. and Lin S.Y. (2009): A preliminary investigation of the association between serum uric acid and impaired renal function. Chang Gung Medical Journal 32 (1): 66-71.

Christakos S., Dhawan P., Porta A., Mady L. and Seth T.(2011): Vitamin D and intestinal calcium absorption. Molecular and Cellular Endocrinology 347(1-2): 25-29.

Crew K.D. (2013): Vitamin D: are we ready to supplement for breast cancer prevention and treatment?. International Scholarly Research Notices 22 pages.

Cunningham J., Locatelli F. and Rodriguez M. (2011): Secondary hyperparathyroidism: pathogenesis, disease progression and therapeutic options. Clinical Journal of the American Society of Nephrology 6: 913-921.

Cynda A.J., Andrew S.L., Josef C., Adeera L., Joseph L. and Garabed E. (2004): Clinical practice guidelines for chronic kidney disease in adults: part 1. Definition, disease stages, evaluation, treatment and risk factors. American Family Physicians. 2004 sep

Dalgard C., Petersen M.S., Weihe P. and Grandjean P. (2011): Vitamin D status in relation to glucose metabolism and type 2 diabetes in Septuagenarians. Journal of Diabetes Care (6): 1284-1288.

de Boer I., Katz R. and Chonchol M. (2011): Serum 25-hydroxyvitamin D and change in estimated glomerular filtration rate. Clinical Journal of the American Society of Nephrology 6 (9): 2141-2149.

de Boer I.H., Ioannou G.N., Kestenbaum B., Brunzell J.D. and Weiss N.S. (2007): 25-Hydroxyvitamin D levels and albuminuria in the Third National Health and Nutrition Examination Survey (NHANES III). American Journal of Kidney Diseases 50: 69-77.

de Zeeuw D., Agarwal R., Amdahl M., Audhya P., Coyne D. and Garimella T., et al. (2010): Selective vitamin D receptor activation with paricalcitrol for reduction of albuminuria in patients with type 2 diabetes (VITAL study): a randomized controlled trial. Lancet 376 (9752): 1543-1551.

Deeb K.K., Trump D.L. and Johnson C.S. (2007): Vitamin D signalling pathways in cancer: Potential for anticancer therapeutics. Nature Reviews Cancer 7: 684-700.

Djalali M., Taheri E., Saedisomeolia A., Djazayeri A., Rahemi A., Hashemi M. and Larijani B. (2013): Vitamin D status of type 2 diabetic patients compared with healthy subjects in the Islamic Republic of Iran. Journal of Eastern Mediterranean Health (9): 1-6.

Drake M.T., Maurer M.J., Link B.K., Habermann T.M., Ansell S.M., Micallef I.N., Kelly J.L., Macon W.R., Nowakowski G.S. and Inwards D.J. (2010):

Vitamin D insufficiency and prognosis in non-Hodgkin's lymphoma. Journal of Clinical Oncology (28): 4191-4198.

Druml W., Metnitz B., Schaden E., Bauer P. and Metnitz P.G. (2010): Impact of body mass on incidence and prognosis of acute kidney injury requiring renal replacement therapy. Intensive Care Medicine 36: 1221-1228.

Drey N., Roderick P., Mullee M. and Rogerson M. (2003): A population-based study of the incidence and outcomes of diagnosed chronic kidney disease. American Journal of Kidney Diseases 42 (4): 677-684.

Dusso A., González E.A. and Martin K.J. (2011): Vitamin D in chronic kidney disease; Best Practice and Research Clinical Endocrinology and Metabolism 25: 647-655.

Eckardt K.U., Berns J.S., Rocco M.V. and Kasiske B.L. (2009): Definition and classification of CKD: the debate should be about patient prognosis--a position statement from KDOQI and KDIGO. American Journal of Kidney Diseases 53 (6): 915-920.

Ejerblad E., Fored C.M., Lindblad P., Fryzek J., McLaughlin J.K. and Nyren O. (2006): Obesity and risk for chronic renal failure. Journal of the American Society of Nephrology 17(6): 1695-1702.

Elhenawe A.R. (2014): Serum vitamin D level In coronary artery disease patients from Gaza Strip. Master Thesis, The Islamic University of Gaza, Gaza Strip, Palestine.

Faratro R., D'Gama C., Fung S. and Tantalo R. (2004): Nocturnal home hemodialysis clinic: Home hemodialysis manual, Toronto, Ontario.

Fawcett J.K. and Scott J.E. (1960): A rapid and precise method for the determination of urea. Journal of Clinical Pathology 13(2): 156-159.

Fleet J.C., Desmet M., Johnson R. and Li Y. (2012): Vitamin D and cancer: A review of molecular mechanisms. Biochemical Journal 441: 61-76.

Freedman D.M., Looker A.C., Chang S.C. and Graubard B.I. (2007): Prospective study of serum vitamin D and cancer mortality in the United States. Journal of the National Cancer Institute 99: 1594-1602.

Fossati, P., Prencipe L. and Berti G. (1980): Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/ 4-aminophenazone chromogenic system in direct enzymatic assay of uric acid in serum and urine. Clinical Chemistry 26: 227-231.

Gansevoort R.T., Matsushita K., van der Velde M., Astor B.C., Woodward M. and Levey A.S. (2011): Lower estimated GFR and higher albuminuria are associated with adverse kidney outcomes. A collaborative meta-analysis of general and high-risk population cohorts. Kidney International 80: 93-104.

Gelber R.P., Kurth T., Kausz A.T., Manson J.E., Buring J.E., Levey A.S. and Gaziano J.M. (2005): Association between body mass index and CKD in apparently healthy men. American Journal of Kidney Diseases (46): 871-880.

Gonzalez-Molero I., Rojo-Martinez G., Morcillo S., Gutierrez-Repiso C., Rubio-Martin E. and Almaraz M.C. (2012): Vitamin D and incidence of diabetes: A Prospective Cohort Study. Journal of Clinical Nutrition 4: 571-573.

Gouda Z., Mashaal G., Bello A.K., El Attar A., El Kemmry T., El Reweny A. and El Nahas M. (2011): Egypt information, prevention, and treatment of chronic kidney disease (EGIPT-CKD) program: prevalence and risk factors for microalbuminuria among the relatives of patients with CKD in Egypt. Saudi Journal of Kidney Diseases and Transplantation 22 (5): 1055-1063.

Grober U., Spitz J., Reichrath J., Kisters K. and Holick M. (2013): Vitamin D: Update 2013 From rickets prophylaxis to general preventive healthcare. Dermato-Endocrinology 5: 3.

Gröber U. (2014): Arzneimittel und Mikronährstoffe. korrigierte Auflage, Wissenschaftliche Verlagsgesellschaft, Stuttgart.

Guh J.Y. (2010): Proteinuria versus albuminuria in chronic kidney disease. Nephrology 15: 53-56.

Gutierrez O., Isakova T., Rhee E., Shah A., Holmes J. and Collerone G. (2005): Fibroblast growth factor- 23 mitigates hyperphosphatemia but accentuates calcitriol deficiency in chronic kidney disease. Journal of the American Society of Nephrology 16: 2205-2215.

Guyton A.C. and Hall J.E. (2011): Textbook of Medical Physiology, 12th Edition, Saunders, Philadelphia, USA.

Harward D.H., Bomback A.S., Jennette C.E., Amamoo M.A. and Falk R. (2009): The kidney education outreach program's community-based screenings: participants' demographics and screening results. North Carolina Medical Journal 70 (6): 507-512.

Holden R.M., Morton A.R., Garland J.S., Pavlov A., Day A.G. and Booth S.L. (2010): Vitamins K and D status in stages 3–5 chronic kidney disease. Clinical Journal of the American Society of Nephrology 5 (4): 590-597.

Holick M.F. (2005): The vitamin D epidemic and its health consequences. Journal of Nutrition 135: 2739-2748.

Holick M.F. (2007): Vitamin D deficiency. The New England Journal of Medicine 357 (3): 266-281.

Holick M.F. (2011): Vitamin D: evolutionary, physiological and health perspectives. Current Drug Targets 12 (1): 4-18.

Hossein-Nezhad A. and Holick M.F. (2013): Vitamin d for health: a global perspective. Mayo Clinic Proceedings 88: 720- 755.

Hsu C-y., Iribarren C., McCulloch C.E., Darbinian J. and Go A.S. (2009): Risk factors for end-stage renal disease: 25-year follow-up. Archives of Internal Medicine 169: 342-350.

Huda M.N., Alam K.S. and Harun-Ur-Rashid. (2012): Prevalence of chronic kidney disease and its association with risk factors in disadvantageous population. International Journal of Nephrology 267329.

Hyun Y.Y., Kim H. and Lee K.B. (2014): Fat mass gain predicts estimated GFR decline in a relatively healthy Korean population. Nephron clinical practice 126 (1): 90-96.

Inaguma D., Nagaya H. and Hara K., et al. (2008): Relationship between serum 1,25-dihydroxyvitamin D and mortality in patients with pre-dialysis chronic kidney disease. Clinical and Experimental Nephrology 12 (2): 126-131.

Inserra F., de la Llave G., Alpino M., Castagna R., de la Fuente I., Dorado E., Norbis M., Pinelli L., Puddu M., Santos J.C., Vivas N. and Marelli C. (2007): Survey of risk factors and renal disease in first-degree relatives of dialysis patients. Medicina 67 (1): 8-18.

International Society of Nephrology (2013): Definition and Classification of CKD. Kidney International Supplements 3: 5-14.

Isakova T., Gutierrez O.M., Patel N.M., Andress D.L., Wolf M. and Levin A. (2011): Vitamin D deficiency, inflammation, and albuminuria in chronic kidney disease: complex interactions. Journal of Renal Nutrition 21: 295-302.

Iseki K., Ikenuya Y. and Kinjo K. (2004): Body mass index and the risk of development of end-stage renal disease in a screened cohort. Kidney International 65: 1870-1879.

Ishizaka N., Ishizaka Y., Toda E., Koike K., Seki G. and Nagai R. (2007): Association between obesity and chronic kidney disease in Japanese:

Differences in gender and hypertensive status. Hypertension Research 30 (11): 1059-1064.

Joergensen C., Reinhard H. Schmedes A., Hansen P.R., Wiinberg N., Petersen C.L., Winther K., Parving H.H., Jacobsen P.K. and Rossing P. (2012): Vitamin D levels and asymptomatic coronary artery disease in type 2 diabetic patients with elevated urinary albumin excretion rate. Journal of Diabetes Care (35): 168-172.

Johnson A.M., Rohlfs E.M., Silverman L.M., Burtis C.A. and Ashwood E.R. (1999): Editors Tietz textbook of clinical chemistry. 3rd edition, Philadelphia: W.B. Sounders company. pp 477-540.

Johnson D. (2012): Risk factors for early chronic kidney disease. kidney Health Australia, CARI Guidelines pp (1-41).

Johnson D.W., Atai E., Chan M., Phoon R.K.S., Scott C., Toussaint N.D., Turner G.L., Usherwood T. and Wiggins K.J. (2013): KHA-CARI Guideline: Early chronic kidney disease: Detection, prevention and management. Nephrology 18: 340-350.

Kalantar-Zadeh K. (2008): Clinical Outcomes of Hypocalcemia in Chronic Kidney Disease. Touch Briefings: 19-25.

Kannan S. and Lim H.W. (2014): Photoprotection and vitamin D: a review. Photodermatology Photoimmunology Photomedicine (30): 137-145.

Kendrick J., Cheung A.K., [...], and HOST (Homocysteine Study) Investigators (2012): Associations of plasma 25-Hydroxyvitamin D and 1,25-Dihydroxyvitamin D concentrations with death and progression to maintenance dialysis in patients with advanced kidney disease. American Journal of Kidney Diseases 60 (4): 567-575.

Khader I.M., Snouber S., Alkhatib A., Nazzal Z. and Dudin A. (2013): Prevalence of patients with end stage renal disease on dialysis in the West

Bank, Palestine. Saudi Journal of Kidney Diseases and Transplantation 24 (4): 832-837.

Kienreich K., Tomaschitz A., Verheyen N., Pieber T., Gaksch M., Grübler M. and Pilz S. (2013): Vitamin D and Cardiovascular Disease. Nutrients 5 (8): 3005-3021.

Kim C.S. and Kim S.W. (2014): Vitamin D and chronic kidney disease. The Korean Journal of Internal Medicine 29: 416-427.

Kim S.M., Choi H.J. and Lee J.P. (2014): Prevalence of vitamin D deficiency and effects of supplementation with cholecalciferol in patients with chronic kidney disease. Journal of Renal Nutrition (24): 20-25.

Kovesdy C.P., Ahmadzadeh S., Anderson J.E. and Kalanar-Zadeh K. (2008): Association of activated vitamin D treatment and mortality in chronic kidney disease. Archives of Internal Medicine 168 (4): 397-403.

Kramer H. (2006): Obesity and chronic kidney disease. Contributions to Nephrology 151: 1-18.

Krishnan A.V. and Feldman D. (2011): Mechanisms of the anti-cancer and anti-inflammatory actions of vitamin D. Annual Review of Pharmacology and Toxicology 51: 311-336.

Ku Y.C., Liu M.E., Ku C.S., Liu T.Y. and Lin S.L. (2013): Relationship between vitamin D deficiency and cardiovascular disease. World Journal of Cardiology 5: 9: 337-346.

Kuro-o M. (2011): Klotho and the aging process. The Korean Journal of Internal Medicine 26: 113-122.

Kwan B.C., Kronenberg F., Beddhu S. and Cheung A.K. (2007): Lipoprotein metabolism and lipid management in chronic kidney disease. Journal of the American Society of Nephrology 18: 1246.

Laliberté F., Bookhart B.K. and Vekeman F. (2009): Direct all-cause health care costs associated with chronic kidney disease in patients with diabetes and hypertension: a managed care perspective. Journal of Managed Care Pharmacy 15 (4): 312-322.

Lappe J.M., Travers-Gustafson D., Davies K.M., Recker R.R. and Heaney R.P. (2007): Vitamin D and calcium supplementation reduces cancer risk: Results of a randomized trial. The American Journal of Clinical Nutrition 85: 1586-1591.

Lavie C., Lee J. and Milani R. (2011): Vitamin D and cardiovascular disease will it live up to its hype?. Journal of the American College of Cardiology 58: 1547-1556.

Levey A.S. (2002): Non diabetic Kidney Disease. The New England Journal of Medicine 347: 1505-1511.

Levey A.S., Atkins R. and Coresh J. (2007): Chronic kidney disease as a global public health problem: approaches and initiatives: a position statement from Kidney disease improving global outcomes. Kidney International 72 (3): 247-259.

Levey A.S. and Coresh J. (2012): Chronic kidney disease. Lancet 379: 16.

Levey A.S., de Jong P.E. and Coresh J. (2011): The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. Kidney International 80: 17

Levey A.S., Eckardt K.U., Tsukamoto Y., Levin A., Coresh J. and Rossert J. (2005): Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney International 67: 2089-2100.

Li Y.C. (2013): Vitamin D in Chronic Kidney Disease 180: 98-109.

Mader S. (2004): Mader understanding human anatomy & physiology, 5th ed. 323-340.

Mann C.J. (2003): Observational research methods. Research design II: cohort, cross sectional, and case-control studies. Emergency Medicine Journal (20): 54-60.

Manson J.E., Mayne S.T. and Clinton S.K. (2011): Vitamin D and prevention of cancer—Ready for prime time? The New England Journal of Medicine 364: 1385-1387.

Mansuri S., Badawi A., Kayaniyil S., Cole D., Harris S., Mamakeesick M., Maguire J., Zinman B. and Hanley A. (2014): Associations of total, bioavailable, and free 25(OH)D concentrations with insulin resistance and beta cell function in an Aboriginal Canadian community. The Journal of the Federation of American Societies for Experimental Biology 28: Supplement 628.5.

Marieb E. (2003): Essentials of human anatomy and physiology, 7th ed. USA p: 479-501.

Maryam Tohidi., Mitra Hasheminia., [...]. and Farzad Hadaegh. (2012): Incidence of chronic kidney disease and its risk factors, Results of Over 10 Year Follow Up in an Iranian Cohort. Plos.

Masoud A.D. (2014): Serum vitamin D level in type 2 diabetic patients from Gaza Strip. Master Thesis, The Islamic University of Gaza, Gaza Strip, Palestine.

Mathisen U., Melsom T., Ingebretsen O.C., Jenssen T.G., Njølstad I., Solbu M.D., Toft I. and Eriksen B.O. (2010): The relationship between glomerular filtration rate measured as iohexol-clearance and ambulatory blood pressure in the general population. Journal of Hypertension 28: 159.

Matias P.J., Jorge C. and Ferreira C. (2010): Cholecalciferol supplementation in hemodialysis patients: effects on mineral metabolism, inflammation, and cardiac dimension parameters. Clinical Journal of the American Society of Nephrology 5 (5): 905-911.

McClellan W.M., Schoolwerth A.C. and Gehr T. (2006): Clinical management of chronic kidney disease. 3:37.

McIntyre N.J., Fluck R.J., McIntyre C.W. and Taal M.W. (2011): Skin autofluorescence and the association with renal and cardiovascular risk factors in chronic kidney disease stage 3. Clinical Journal of the American Society of Nephrology 14: 2356-2363.

Mehrotra R., Kermah D.A. and Salusky I.B. (2009): Chronic kidney disease, hypovitaminosis D, and mortality in the United States. Kidney International 76: 977-983.

Ministry of Health (MOH), (2005): Annual report. Palestine.

Ministry of Health (2010): Health Annual Report 2009, Palestinian Health Information Center, Palestinian National Authority.

Motlagh B., O'Donnell M. and Yusuf S. (2009): Prevalence of cardiovascular risk factors in the Middle East: a systematic review. The European Journal of Cardiovascular Prevention and Rehabilitation 16 (3): 268-280.

Nakai S., Watanabe Y. and Masakane I.B. (2013): Overview of regular dialysis treatment in Japan (as of December 31, 2011). Therapeutic Apheresis and Dialysis 17: 567.

National Health Service (2010): Palestinian Health Information Center (PHIC) Ministry of Health-Palestine (MOH), Annual report. 2005.

National Kidney Foundation (NKF). (2002): K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. American Journal of Kidney Diseases 39 (2 Suppl 1): S1-266.

National Kidney Foundation (2007): Guidelines and Commentaries, Kidney Disease, About Chronic Kidney Disease.

National Kidney Foundation (2013): Guidelines and Commentaries, Kidney Disease, About Chronic Kidney Disease.

Newman D.J. and Price C.P. (1999): Renal function and nitrogen metabolites. In: Burtis C.A, Ashwood E.R, (editors) Text book of clinical chemistry. 3rd edition, Philadelphia: W.B Standers Company. pp 1204-1207.

Nigwekar S.U., Bhan I. and Thadhani R. (2012): Ergocalciferol and cholecalciferol in CKD. American Journal of Kidney Diseases 60: 139-156.

Nigwekar S.U., Tamez H. and Thadhani R.I. (2014): Vitamin D and chronic kidney disease–mineral bone disease (CKD–MBD). Official Journal of the International Bone and Mineral Society 498.

Nugent R.A., Fathima S.F., Feigl A.B. and Chyung D. (2011): The burden of chronic kidney disease on developing nations: a 21st century challenge in global health. Nephron Clinical and Practice 118 (3): 269-277.

Obi Y., Hamano T. and Isaka Y. (2015): Prevalence and prognostic implications of vitamin D deficiency in chronic kidney disease. Hindawi Publishing Corporation Disease Markers. p1-9.

Ohta M., Babazono T. and Uchigata Y. (2010): Comparison of the prevalence of chronic kidney disease in Japanese patients with Type 1 and Type 2 diabetes. Diabetic Medicine 27 (9): 1017-1023.

Ozfirat Z. and Chowdhury T. (2010): Vitamin D deficiency and type 2 diabetes. Journal of Postgrad Medicine 86: 18-25.

Palestinian Clinical Laboratory Tests Guide (PCLTG), (2005): Ministry of Health- Palestine (MOH), first edition.

Palomer X., Gonza´lez-Clemente J.M., Blanco-Vaca F. and Mauricio D. (2008): Role of vitamin D in the pathogenesis of type 2 diabetes mellitus . Journal of Diabetes. Obesity and Metabolism 10: 185-197.

Pilz S., Tomaschitz A., Friedl C., Amrein K. and Drechsler C. (2011): Vitamin D status and mortality in chronic kidney disease. Nephrology Dialysis Transplantation 26: 3603-3609.

Prietl B., Treiber G., Pieber T. and Amrein K. (2013): Vitamin D and immune function. Nutrients 5: 2502-2521.

Rao A.M., Bitla A.R., Reddy E.P., Sivakumar V. and Srinivasa Rao P.V.L.N. (2010): Lipid abnormalities, lipoprotein (a) and apoprotein pattern in non-dialyzed patients with chronic kidney disease. Indian Journal of Clinical Biochemistry 25 (1): 47-50.

Ravani P., Malberti F., Tripepi G., Pecchini P., Cutrupi S. and Pizzini P. (2009): Vitamin D levels and patient outcome in chronic kidney disease. Kidney International 75 (1): 88-95.

Reese R.W. (2006): Vitamin D and bone health. Journal of Lancaster General Hospital 1: 78-87.

Renal Resource Center, (2010): Understanding chronic kidney disease. The Austuralian and Newzeland Society of Nephrology, Northern Sydney Central Cost – NSW Health.

Robsahm T., Schwartz G. and Tretli S. (2013): The inverse relationship between 25-Hydroxyvitamin D and cancer survival: Discussion of Causation. Cancers 5: 1439-1455.

Rojas-Rivera J., De La Piedra C., Ramos A., Ortiz A. and Egido J. (2010): The expanding spectrum of biological actions of vitamin D. Nephrology Dialysis Transplantation 25 (9): 2850-2865.

Rozita M., Afidza M.N., Ruslinda M., Cader R., Halim A.G., Norella Kong C.T., Azmi K.N. and Shah S.A. (2013): Serum vitamin D levels in patients with chronic kidney disease. EXCLI Journal 12: 511-520.

Sabbagh Z., Markland J. and Vatanparast H. (2013): Vitamin D status is associated with disease activity among rheumatology outpatients. Nutrients 5: 2268-2275.

Sai A.J., Walters R.W., Fang X. and Gallagher J.C. (2011): Relationship between Vitamin D, Parathyroid Hormone, and Bone Health. The Journal of Clinical Endocrinology and Metabolism 96 (3): 436-446.

Satirapoj B., Limwannata P., Chaiprasert A., Supasyndh O. and Choovichian P. (2013): Vitamin D insufficiency and deficiency with stages of chronic kidney disease in an Asian population. BMC Nephrology 206 (14): 1471-2369.

Scragg R., Sowers M. and Bell C. (2007): Serum 25-hydroxyvitamin D, ethnicity, and blood pressure in the Third National Health and Nutrition Examination Survey. American Journal of Hypertension 20: 713-719.

Shin S.Y., Kwon M.J., Park H. and Woo H.Y. (2014): Comparison of chronic kidney disease prevalence examined by the chronic kidney disease epidemiology collaboration equation with that by the modification of diet in renal disease equation in Korean adult population. Journal of Clinical Laboratory Analysis. Feb 27

Shoben A.B., Rudser K.D., de Boer I.H., Young B. and Kestenbaum B. (2008): Association of oral calcitriol with improved survival in nondialyzed CKD. Journal of the American Society of Nephrology 19 (8): 1613-1619.

Song J.W. and Chung K.C. (2010): Observational Studies: Cohort and Case-Control Studies. Plast Reconstr Surg. 126 (6): 2234-2242.

Stenvinkel, (2010): Chronic kidney disease: a public health priority and harbinger of premature cardiovascular disease. Journal of Internal Medicine 26 (8): 456-467.

Stevens P.E., O'Donoghue D.J., de Lusignan S., Van Vlymen J., Klebe B., Middleton R., Hague N., New J. and Farmer C.K. (2007): Chronic kidney disease management in the United Kingdom: NEOERICA project results. Kidney International 72 (1): 92-99.

Stivelman E. and Retnakaran R. (2012): Role of vitamin D in the pathophysiology and treatment of type 2 diabetes. Current Diabetes Reviews 8: 42-47.

Stringer S., Sharma P., Dutton M., Jesky M., Ng K., Kaur O., Chapple I., Dietrich T., Ferro C. and Cockwell P. (2013): The natural history of, and risk factors for, progressive Chronic Kidney Disease (CKD): the Renal Impairment in Secondary care (RIISC) study; rationale and protocol. BMC Nephrology 14: 95.

Stubbs J.R., Idiculla A., Slusser J., Menard R. and Quarles L.D. (2010): Cholecalciferol supplementation alters calcitriol responsive monocyte proteins and decreases inflammatory cytokines in ESRD. Journal of the American Society of Nephrology 21 (2): 353-361.

Takahashi N., Udagawa N. and Suda T. (2014): Vitamin D endocrine system and osteoclasts**.** International Bone & Mineral Society**.** BoneKEy Reports 3, Article number: 495 .

Takiishi T., Belle T., Gysemans C. and Mathieu C. (2013): Effects of vitamin D on antigen-specific and non-antigen-specific immune modulation: relevance for type 1 diabetes. Pediatric Diabetes 14 (2): 81-148.

Targher G., Bertolini L., Padovani R., Zenari L., Scala L., Cigolini M. and Arcaro G. (2006): Serum 25-hydroxyvitamin D3 concentrations and carotid artery intima-media thickness among type 2 diabetic patients. Clinical Endocrinology (Oxf) 65: 593-597.

Tesauro M., Canale M.P. and Rodia G. (2011): Metabolic syndrome, chronic kidney, and cardiovascular diseases: role of adipokines. Cardiology Research and Practice Article ID 653182.

Thibodeau G. and Patton K. (1999): Anatomy and physiology, urinary system, chapter 28, fourth edition, Mosby, 823-825.

The Hashemite kingdom of Jordan, Ministry of Health, (2008): Non communicable disease directorate, National Registration of ESRD, Annual Report 2008.

Thomas L. (1998): Clinical Laboratory Diagnostics. 1st edition. Frankfurt: THBook Verlaagsgesellschaft. pp: (374-7).

Tiez N.W. (1994): Speciman Collection and Processing; Sources of Biological Variation. Textbook of Clinical Chemistry, 2nd Edition, WB. Saunders, Philadephia, PA.

Tracy S.M. and Mazen J.H. (2010): The role of vitamin D deficiency in the pathogenesis of type 2 diabetes mellitus. European Journal of Clinical Nutrition and Metabolism 5: 155-165.

Tsuruya K., Yoshida H., Nagata M., Kitazono T., Hirakata H., Iseki K., Moriyama T., Yamagata K., Yoshida H., Fujimoto S., Asahi K., Kurahashi I., Ohashi Y. and Watanabe T. (2014): Association of the triglycerides to high-density lipoprotein cholesterol ratio with the risk of chronic kidney disease: analysis in a large Japanese population. Atherosclerosis 233 (1): 260-267.

Urena-Torres P., Metzger M. and Haymann J.P. (2011): Association of kidney function, vitamin D deficiency, and circulating markers of mineral and bone disorders in CKD. American Journal of Kidney Diseases 58: 544-553.

Usatii M., Rousseau L. and Demers C. (2007): Parathyroid hormone fragments inhibit active hormone and hypocalcemia-induced 1,25(OH)2D synthesis. Kidney International 72: 1330-1335.

U.S. Renal Data System. USRDS (2009): Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD; 2009.

Vanga S.R., Good M., Howard P.A. and Vacek J.L. (2010): Role of vitamin D in cardiovascular health. American Journal of Cardiology 106 (6): 798-805.

Vijayakumar M., Nammalwar B. and Prahlad N. (2007): Prevention of chronic kidney disease in children. Indian Journal of Nephrology 17 (2): 47-52.

Virtanen J.K., Nurmi T., Voutilainen S., Mursu J. and Tuomainen T.P. (2011): Association of serum 25-hydroxyvitamin D with the risk of death in a general older population in Finland. European Journal of Nutrition 50: 305-312.

Wacker M. and Holick M. (2013): Vitamin D effects on skeletal and extraskeletal health and the need for supplementation. Nutrients 5 (1): 111-148.

Wang C. (2013): Role of vitamin D in cardiometabolic diseases. Journal of Diabetes Research :http://dx.doi.org/10.1155/2013/243934.

Wang Y., Chen X., Song Y., Caballero B. and Cheskin L.J. (2008): Association between obesity and kidney disease: a systematic review and meta-analysis. Kidney International 73 (1): 19-33.

Warady and Chadha, 2007: Chronic kidney disease in children: the global perspective. Pediatric Nephrology 22 (12): 1999-2009.

Ward M.M. (2008): Socioeconomic status and the incidence of ESRD. American Journal of Kidney Diseases 51: 563-572.

Wen C.P., Cheng T.Y and Tsai M.K. (2008): All-cause mortality attributable to chronic kidney disease: A prospective cohort study based on 462 293 adults in Taiwan. Lancet 371: 2173-2182.

White S.L., McGeechan K. and Jones M. (2008): Socioeconomic disadvantage and kidney disease in the United States, Australia, and Thailand. American Journal of Public Health 98: 1306-1313.

Wickman C. and Kramer H. (2013): Obesity and kidney disease: Potential Mechanisms. Seminars in Nephrology 33: 14-22.

Wiggins K. and Johnson D. (2012): Symptoms, natural history and outcomes of early chronic kidney disease. Kidney Health Australia, CARI GUIDELINES: 1-12.

Williams S., Malatesta K. and Norris K. (2009): Vitamin D and chronic kidney disease. Ethnicity & Disease 19 (4 Suppl 5): S5-8-11.

Wolf M., Shah A., Gutierrez O., Ankers E., Monroy M. and Tamez H. (2007): Vitamin D levels and early mortality among incident hemodialysis patients. Kidney International 72: 1004-1013.

World Health Oragnization, WHO. (2012): Ten facts on obesity, edited. Available on: http://www.who.int/features/factfiles/obesity/facts/en/index.html (accessed 12 Nov 2012).

Ying H-Q., Sun H-L., He B-S., Pan Y-Q., Wang F., Qi-Wen Deng Q-W., Chen J., Liu X. and Wang S-K. (2014): Circulating vitamin D binding protein,

total, free and bioavailable 25-hydroxyvitamin D and risk of colorectal cancer. Scientific Reports 5 : (1-5).

Yokoyama H., Sone H., Oishi M., Kawai K., Fukumoto Y. and Kobayashi M. (2009): Prevalence of albuminuria and renal insufficiency and associated clinical factors in type 2 diabetes: the Japan Diabetes Clinical Data Management study (JDDM15). Nephrology Dialysis Transplantation 24: 1212-1219.

Zhang L., Wang F. and Wang L. (2012): Prevalence of chronic kidney disease in China: a cross-sectional survey. Lancet 15: 815-822.

Zhang R. and Naughton D.P. (2010): Vitamin D in health and disease: Current perspectives. Nutrition Journal 9 (65).