Sudan University of Science and Technology

College of Graduate Studies

Assessment of serum Testosterone and PRL Levels among Males Chronic Kidney Disease Patients in Khartoum State

تقييم مستويات التستسترون والبرولاكتين في مصل المرضى الذكور المصابين بمرض الفشل الكلوي في ولاية الخرطوم

A Dissertation Submitted in Partial Fulfillment for the Requirement of M.Sc Degree in Medical Laboratory Science (Clinical Chemistry)

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Nov, 2018

الآية

قال تعالى :

﴿ إِنَّمَا أَمْرُهُ إِذَا أَرَادَ شَيْئًا أَنْ يَقُولَ لَهُ كُنْ فَيَكُونُ ﴾

سورة يس الآية : (82)

Dedication

Every challenging work needs self-efforts as well as guidance of elders especially those whom were very close to our heart.

My humble effort I dedicate to my lovely family whom taught me patience, strife, and pushed.

Me towards success in life and give me all care and happiness.
Along with all hard working and respected teachers

Acknowledgements

First of all, I thank God for all the beneficent and most merciful. I am very thankful to my supervisor ***Dr: Abdelkrim Abu Bakr*** *for his help and guidance. I feel thankful to him for his insightful advice and suggestions.*

In addition, special thanks to the teaching staff of clinical chemistry department and to all of my colleagues in Medical laboratory science Sudan University of science & technology.

I found myself very lucky that I got very kind people. In particularly, I am thankful to ***Ramy*** *(Modern Medical Centre) hospital special thanks for their effort, kindness, and support in lab work.*

I would like also to express my thanks ***Mojtaba ahmed ,Mhmed Ahmedand Muntaser ahmed*** *for the great effort and time that spent in my research.*

ABSTRACT

Background: chronic kidney disease is recognized as a major health problem standing the community over years. Furthermore, it affects most population under different aging with prevalence between 11% - 13% globally and 5%-7% in Sudan. Additionally, it is defined by persistent urine abnormalities, structural abnormalities or impaired excretory renal function suggestive of loose of functional nephron. Hence, the aim of this study was to investigate the association of the serum testosterone and Prolactin hormones in patients with chronic kidney disease.

Methods: This case control study was conducted at Alturkey hospital and Ultra lab with in three months from September 2018 to November 2018, the study carried out on a total sample of 80 including 40 chronic kidney disease patients as case and 40 healthy individual as control group. Serum prolactin levels was estimated using Enzyme linked immune sorbent assay technique and serum testosterone level was estimated using TOSOH AIA 1800 system analyzer. Data analyzed using SPSS soft ware program version 21.

Results: This study showed that the levels of testosterone was significantly decreased with (p-value 0.000); while there was significant elevation in level of prolactin with (p-value 0.000) in Chronic Kidney Disease patients compared to control group. When comparing Chronic Kidney Disease patients according to Diabetus Mellitus , presence of hypertensions and take of medication the study showed that there was significant increase in prolactin and significant decrease in testosterone in diabetic patients compared to non diabetic patients with (p-value 0.038, p-value 0.033) respectively and there was insignificant difference between hypertensive & non hypertensive in the level of prolactin and testosterone in chronic kidney disease patients with (p.value 0.066 p.value 0.440) respectively, also there was insignificant difference between patients had taken medication and

no taken medication in the level of prolactin and testosterone in chronic kidney disease patients with (p.value 0.620 p.value 0.267) respectively.

There was no correlation between testosterone levels with duration, and body mass index (R -0.064 P.value 0.694, R -0.088 P.value 0.588) and negative correlation with age (R -0.562 P.value 0.000) respectively. Also, there was positive correlation between prolactin with duration and body mass index with (R0.726 P.value 0.000 ** R 0.639 P.value 0.000) respectively and no correlation between prolactin and age with (R 0.158 P.value 0.331).

Conclusion: this study concludes that, the patients with Chronic Kidney Disease have high prolactin and low testosterone

المستخلص

الخلفيه: على الصعيد العالمي ، تم التعرف على الفشل الكلوي المزمن باعتباره مشكله صحيه رئيسيه تهدد المجتمع على مدى سنوات في مختلف الاعمار بمعدل انتشار عالمي 11%-13% وفي السودان بمعدل 5%-7%. ويعرف الفشل الكلوي المزمن بتغييرات مستمره في البول او تشوهات في تركيب الكلى ووظائفها الاخراجية التي تشير الي تدهور الوحدة الوظيفية للكلى (النفرون). وكان الهدف من الدراسه لمعرفه تأثير الفشل الكلوي المزمن على هرمونات الخصوبه لدى الذكور.

الطريقه:اجريت هزه الدراسه في مستشفى التركي ومعمل الترا وكانت دراسة الحالات والشواهد من سبتمبر الي نوفمبر2018 ، واجريت الدراسه على عدد 80 شخصا ومن بينهم 40 حاله من مرضى الفشل الكلوي و40 افراد اصحاء كعينات ضابطة، وتم قياس هرمون (برولاكتين) في المصل باستخدام تقنيه (إليزا) ، وقياس هرمون (الستستيرون) في المصل باستخدم نظام محلل (TOSOH AIA 1800) اما تحليل البيانات فقد تم باستخدام برنامج الحزمة الاجتماعية للعلوم الإحصائية إصدار 21.

النتيجة: بينت هذه الدراسه ان هنالك إنخفاض ذو دلاله معنويه في مستويات التيستسترون بقيمه ضابطه 0.000 مع ارتفاع ذو دلاله معنويه في مستويات البرولاكتين بقيمة ضابطه 0.000 فى مرضى الفشل الكلوي المزمن مقارنه بمجموعه التحكم . عندي مقارنة مرضى الفشل الكلوي المقارنه تبعا لمرض السكري، ارتفاع ضغط الدم وتناول الادوية، بينت الدراسة ان هنالك انخفاض في مستويات مع ارتفاع في مستويات البرولاكتين في مرضى السكرى مقارنة بغير مرضى السكرى بقيمه ضابطه (0.033 0.038) على التوالي، وهنالك اختلاف ليس له دلالة معنوية في مستويات التستسترون والبرولاكتين في المرضى المصابين بارتفاع ضغط الدم مقارنة بغير المصابين بقيمه ضابطه (0.066 0.440) على التوالي ،

وايضا ان هنالك اختلاف ليس له دلاله معنوية في مستويات التستسترون والبرولاكتين في المرضى الذين يتناولون الدواء مقارنه بغيرهم.

كما أوضحت الدراسة أن مستويات التستسترون ليس لها علاقة مع مدة المرض ومؤشر كتلة الجسم وعلاقة سلبية ذو دلالة معنوية مع العمر. وأيضاً مستويات البرولاكتين لها علاقة إيجابية ذي دلالة معنوية مع مدة المرض ومؤشر كتلة الجسم وليس لها علاقة مع العمر.

الخلاصة: خلصت هذه الدراسة الى مريض الفشل الكلوي المزمن يعاني من زيادة هرمون البرولاكتين مع إنخفاض مستوي التستسترون.

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Chapter One

Introduction, Objective and Rationale

1.1 Introduction

Chronic kidney disease (CKD) is defined by persistent urine abnormalities, structural abnormalities or impaired excretory renal function suggestive of a loss of functional nephrons (Romagnani *et al*, 2017).

CKD has global prevalencebetween11% - 13% (Hill et al., 2016). Many diseases processes can lead to decrease in the number of functioning nephrons and progressive, Irreversible impairment of renal function, these include: Glomerulonephritis, diabetes mellitus, hypertension, pyelonephritis, Reno-vascular disease and polycystic kidney (Marshall et al, 2012).

In addition, many hormones affected by CKD because it is metabolized or excreted by the kidney (Matthew, 2017).Endocrine abnormalities are a common feature of chronic renal insufficiency, and endocrine dysfunction is proportional to the degree of renal impairment. Changes of androgen synthesis and metabolism develop early after the onset of renal insufficiency and are likely to be caused by primary hypogonadism and/or disturbances of the hypothalamic pituitary axis and impaired function of the hypothalamic pituitary gonadal axis is not reversed by initiation of effective hemodialysis or peritoneal dialysis therapy (Osman and Ismail, 2016).

CKD causes variation of different serum hormones level for example testosterone and thyroid hormones show a decrease level while prolactin and PTH show increase level. Reductions in circulating sex steroid levels may not only lead to clinical hypogonadism, but may also play a role in the pathogenesis and progression of CKD (Osman and Ismail, 2016).

Prolactin is a hormone secreted by various tissues in addition to the anterior pituitary gland. Its biologic action in women is to control breast development and lactation. The role of prolactin in men remains still unclear (Kerstin *et al*., 2017).

Higher serum prolactin concentrations are common in patients with advanced chronic kidney disease and end stage renal disease (ESRD), secondary to increased secretion and, to a lesser extent, reduced metabolic clearance (Joan *et al*., 2017).

It has been reported that, hyperprolactinemia is associated with gynecomastia and sexual dysfunction in male CKD patients. Furthermore, elevated levels of serum prolactin which occurs in CKD may contribute to vascular derangements. This might lead to worse cardiovascular outcomes among CKD patients (Nehru *et al*., 2016).

Testosterone is an anabolic hormone that plays an important role in muscle anabolism (Cigarran *et al.,* 2017). Patients with end-stage renal disease are found to be at increased risk for hypogonadism. In one report, testosterone deficiency (<10 nmol/L) was present in 44% of the men with renal failure, while 33% showed testosterone insufficiency (10–14 nmol/L), and only 23% had normal testosterone values (Thirumavalavan *et al*., 2015). Testosterone deficiency is the most common gonadal alteration in men mainly because of reduced prolactin clearance and uremic inhibition of luteinizing hormone signaling at the level of the Leydig cells (Osman and Ismail, 2016). Moreover, other observation reported that, Low testosterone level is associated with endothelial dysfunction and the possibility of cardiovascular events (Cigarran *et al.,* 2017).

1.2 Rationale:

The incidence of CKD in Sudan is about 33%, increases annually. Patient with CKD as well as they develop CVD, hypertension, and osteoporosis in addition Hypogonadism is present in 26%-66% of men with different stage of renal failure which may negatively affect not only sexual function but also other features of the disease, namely progression to ESRD, body composition, quality of life , muscle function and nutritional status, depression, hypertension, hyperlipidemia, DM and anemia .testosterone deficiency is suggested to take part in atherosclerosis process. Emerging evidence indicates that androgens may provide protective effect against the development and progression of atherosclerosis. In this study we will provide an understanding of the occurrence of abnormalities including hypogonadism and sexual dysfunction that occur secondarily on patients with CKD, then becomes essential to design a rational approach to their prognosis, treatment and to design a new strategy**.**

1.3 Objectives

1.3.1 General objectives

To assess serum testosterone and prolactin level among chronic kidney disease males patients in Khartoum state.

1.3.2Specific objectives

1) To estimate biochemical parameters (testosterone, prolactin) in study group and control group.

2) To compare mean concentration of biochemical parameters (testosterone, prolactin) in case and control group, and parameters with variable in study group.
3) To correlate biochemical parameters with risk factors according to variables (age, BMI, duration of disease).

Chapter Two

Literature Review

Chapter Two

Literature Review

2.1 Kidneys

2.1.1 Anatomy

Kidneys are two bean-shaped organs located at the back of the abdominal cavity is the retroperitoneal space, It is about 4.5 inches long and 2.5 inches wide, kidneys are highly vascular receive blood from the paired arteries exits into the paired renal veins. Each kidney is attached to ureter, a tube that carries excreted urine to the bladder (Cotran *et al*., 2005 and Cindy, 2017).

There are about one million nephrons per kidney, each of which is made up of five main functional segments: the glomeruli, proximal convoluted tubules and distal convoluted tubules in cortex of kidney, loop of Henle and collecting duct in renal medulla (Martin, 2013).

2.1.2 Kidney function:

The kidneys serve important functions including filtration and excretion of metabolic waste product (urea and ammonium), regulation of electrolytes, and acid-base balance, kidney also control the reabsorption of water to maintain intravascular volume. In addition, it has hormonal function via Vitamin D activation, erythropoietin production and serve to regulate blood pressure via RAAS (Charbel and Thomas, 2017).

2.1.3 Kidney physiology:

The formation of urine occurs through three steps:

1) Glomerular filtration that filter out most of the wastes which can be achieved by difference between the mean transcapillary hydrostatic pressure and the mean transcapillary oncotic pressure.

2) Tubular reabsorption depend on low blood pressure in tubule and energy dependent

transport, tubules contribute to fluid, electrolyte, and nutrient homeostasis by reabsorbing approximately 60%-70% of water, NaCl and nearly all nutrients. 3) Tubular secretion where some hormone and electrolytes are secreted (Marke *et al*., 2016).

2.1.4 The role of kidneys in hormone clearance:

The kidneys play a significant part in maintaining homeostasis in an organism. They participate in the excretion of various hormones: cortisol, aldosterone, sex hormones, thyroid gland hormones, and catecholamine. Also, in biodegradation of peptide hormones such as parathyroid hormone, calcitonin and insulin. In patients with uremia, impairment of hormone excretion and biodegradation has been observed, as well as disorders affecting excretion, transportation and binding hormones with target cells, frequently as a result of receptor resistance. Of kidney in hormone clearance (Stanislaw *et al*., 2012).

2.1.5 Kidney disease

Occur when biological function of kidney such as" excretion, homeostatic regulation and endocrine function, Mechanism of differential reabsorption and secretion that are located in the tubule of nephron" are disturbed. The kidneys have considerable ability to increase their functional capacity in response to injury. Thus a 50% to 60% reduction in the functioning renal mass may occur before the onset of any significant symptoms or even before any major biochemical alteration appears. So when the excretory function of the kidneys declines over hours or days refers to suggest diagnosed by AKI that are common condition complicating 5% of hospital admission. The evidence gathered from in vitro studies suggest that filtration of an abnormal amount or type of protein by the damaged glomerulus may induce mesangial cell injury,

leading to glomerulosclerosis, and that some proteins also have an adverse effect on proximal tubular cell function. Nephrons are lost via: toxic, anoxic, immunological injury that may initially injure the glomerular tubule or both (Burtis *et al*., 2010).

Glomerular damage involves endothelial or mesangial cells or basement membrane so is according to the onset and duration of disease into the following sections:

2.1.6 Acute kidney injury

Is a sudden, sharp decline in renal function as a result of an acute toxic or hypoxic insult to the kidney, defined as occurring when the GFR is reduced to less than 10 ml/min. this syndrome is subdivided into three types depending on the location of the precipitating defect into pre-renal, renal and post renal (Bishop *et al*., 2010).

AKI is characterized by rapid loss of renal function, with retention of urea, creatinine, hydrogen ions and other metabolic products and usual but not always oliguria <400mL urine/24h.Although potentially reversible, the consequences to homeostatic mechanisms are so profound that this condition continues to be associated with a high mortality. Furthermore, AKI often develops in patients who are already severely ill, with multiple organ involvement (Marshall *et al*., 2012).

2.1.7 Chronic Kidney Disease

2.1.7.1 Definition of CKD

CKD is defined as the presence of kidney damage or decreased kidney function that persists for at least three months. Chronic kidney disease is often associated with progressive and irreversible loss of large number, of functioning nephron. Serious clinical symptom usually doesn’t occur until the

numbers of functional nephrons fall to at least 70% – 75% below normal. In fact, relatively normal blood concentrations of most electrolytes and normal body fluid volumes can still be maintained until the number of functioning nephrons decreases below 20% - 25% of normal (John, 2016).

2.1.7.2 Epidemiology of CKD

CKD is worldwide distributed, several recent studies from different parts of the world reported prevalence of CKD between 8%-13% (Pierre *et al*., 2017). Kidney disease often has no symptoms in its early stages and can't be detected until it's very advanced, Mortality of CKD in 2013 in Medicare patient was (117.9/1000) this rate increase with CKD severity and decrease with dialysis and transplantation (NIDDK, 2016).

2.1.7.3 Classification of CKD

The CKD stages are classified based on calculation of the GFR. In the absence of the evidence of renal disease (proteinuria or structural abnormality) value of more than 60ml/min are regarded as normal (Marshall *et al*, 2012; Martin, 2012).

CKD stage	Description	GER (Ml/min/1.73m^2)	Comments
1	Kidney damage with normal or raised GFR	>90	Requires presence of proteinuria, hematuria or other kidney abnormality e.g. on imaging
2	Kidney damage with mildly decreased GFR	60-89	Requires presence of proteinuria, hematuria or other kidney abnormality e.g. on imaging
3a	Moderately decreased GFR	45-59	Increase risk of cardiovascular complication
3b	Moderately decreased GFR	30-44	Many patients asymptomatic
4	Severely decreased GFR	15-29	Most patients symptomatic
5	Established renal failure	<15	Renal replacement therapy usually required

(Marshall *et al*, 2012)

2.1.7.4 Causes of CKD

In most of cases the progression of kidney disease is the result of chronic disease such as diabetes; in case of uncontrolled DM the accumulated glucose in blood inter tissues and is then converted to sorbitol by aldose reductase, in renal tissues where sorbitol dehydrogenase is low or absent sorbitol accumulates causing water to be drown into cell due to increase osmatic pressure impairing tissue function. Hypertension which can damage the glomeruli, obstructed urine flow which lead to urine retention back into the kidney from the bladder (vesicoureteral reflux) and cause damage , kidney disease like polycystic kidney disease, pyelonephritis also can develop to CKD if not treated , kidney artery stenosis which decrease blood flow to kidney, and also certain toxins in fuels, solvent and lead can cause damage

to kidney , congenital or fetal development of kidneys problem and autoimmune disease like systemic lupus erythematous, good pasture syndrome body immune system attack the kidney as foreign tissue (Christian, 2017; Harvey *et al*., 2011; Medline plus, 2017)

2.1.7.5 Risk factors of CKD

CKD has become a serious public health issue, there are currently over 1.4 million patient receiving renal replacement therapy worldwide, because the need to reduce the occurrences of disease and that by reducing people under risk, risk factors such as family history, older and low birth weight are considered to be strong risk factors, moreover smoking, obesity, exposure to heavy metals, excessive alcohol consumption and analgesic medication also constitute risks. Experiencing acute K.I, history of cardiovascular disease, hyperlipidemia and metabolic syndrome can lead by the time to CKD, HCV, HIV infections and malignancy are further risk (Rumeyza, 2013; Verneda and Elisabeth, 2017).

2.1.7.6 Complication of CKD

Chronic kidney disease and its complication can affect all the organs and systems in body. If CKD is detected early, the associated and following complications and the progression to kidney failure can be delayed or even prevented through appropriate intervention. Potential complication may include: fluid retention which could lead to edema in extremities, HBP, pulmonary edema. Sudden rise in potassium levels in blood (hyperkalemia) which could impair heart ability to function and may be life threating (Bishop *et al*., 2010).

Patients also exposed to: anemia due to iron deficiency and lack of erythropoietin which induce RBCs production, metabolic bone disease is also develop because kidneys are responsible for excreting phosphorus from body and processing vitamin D into its active form. High phosphorus and lack of vitamin D cause blood

level of calcium to decrease causing activation of PTH. These and several complex changes cause the development of metabolic bone disease (Pranay and Benjamin, 2017).

Less sever CKD has been recognized as an independent risk factor for CVD and other common condition that affecting the elderly such as impairment in physical function and cognition. Additionally, metabolic and endocrine complication like erectile dysfunction, decrease libido and amenorrhea may occur (KDIGO, 2012).

2.1.7.7 Lab diagnosis of CKD

Standard clinical methods are used to reach diagnosis i.e. history examination and special investigations based on knowledge of common cause of CKD and their manifestation. Not all evaluations are required in all patients, and will be directed by clinical context and resource availability. For most patients the following evaluations are indicated: Reagent strip urinalysis to detect hematuria or pyuria, if positive use microscope to detect RBCs cast or WBCs cast (KDIGO, 2012).

Blood tests measure the level of excess waste traveling through blood stream such as creatinine and urea, these tests used to evaluate glomerular function and assessing GFR, patients with GFR less than 60 ml/min/1.73m2 for duration of three months are diagnosed as having CKD even if there is no present of kidney damage. Serum calcium, phosphate, 25-hydroxyvitamin D, alkaline phosphatase and PTH are used to diagnose renal bone disease. Kidney biopsy: Removing sample of kidney tissue for testing (avail clinical research, 2018; Pradeep, 2017).

2.1.7.8 Treatment of CKD

2.1.7.8.1 Dialysis

Artificially remove waste products and extracellular fluid from blood, in Hemodialysis machine filter waste and excess fluid, in peritoneal dialysis a thin

tube inserted into abdomen and fill it with dialysis solution that absorbs waste and excess fluid, after period of time the dialysis solution drains from body.

Some centers offer nocturnal HD where patients sleep during treatment by slow, low efficiency dialysis. Home HD is conducted in the home environment, 5-6 sessions weekly for 2.5-3h. Control of BP and phosphorous are superior with PD (Henry, 2011).

2.1.7.8.2 Transplantation

Is the most effective form of RRT, in terms of long term survival and is most preferred option for many patients who have or developing ESRD or will be undergoing chronic dialysis therapy. Approximately 30% of patient on dialysis are selected to be placed on waiting list for transplantation. The major problem in organ transplantation is rejection during which the body has an immune response to the transplanted organ, possibly lead to immediately remove organ from recipient. Thus, successful transplantation requires both; preoperative and postoperative assessment following assessment and therapeutic drug management that is necessary to help prevent acute rejection and loss of transplanted kidney. (Guyton and John, 2016; Burtis *et al*, 2008).

2.2 Pituitary Gland

2.2.1 Definition

Endocrine gland pea shape weighing 0.5 gram found in the bottom of hypothalamus at the base of the brain, the hypothesis or pituitary rest up on the hypophysial fossa of the sphenoid bae. It's known as master gland of the body, produce various hormones to regulate homeostasis this gland is embryological and anatomically composed of two different structures**.** (Saishuyoshia *et al.*, 2016; Shannon *et al*., 2013; Wendy and Jean, 2007).

2.2.2 Anatomy

The pituitary develops as two independent structures (anterior and posterior). Anterior pituitary known as the adenohypophysis because it is consist of glandular tissue and posterior pituitary known as the neurohypophysis because it is connected with hypothalamus by neuron and the important to known that posterior part not secrete hormone but store hormone which produce from hypothalamus. Anterior part Constituting three quarters of the adult pituitary gland and this part linked with hypothalamus by portal veins (capillary plexus) (Marshall *et al,* 2012; Richard and Neil, 2012; Martin, 2012).

2.2.3 Function

The pituitary gland controls important body functions and hormonal system with hypothalamus both control the involuntary system which manages the balance of energy, heat and water in body. Body temperature, heartbeat, sleep, hunger and thirst. It is also produce hormone either have direct effect on target organ (GH) or hormones control other hormones glands(TSH).and the production of hormones from pituitarygland control by either realizing and inhibiting hormones produced by hypothalamus or by hormone level in the blood (Health line medical 2015; Marlene 2008; PubMed health 2015).

2.2.4 Clinical Disorder

2.2.4.1 Pituitary Tumor

It is the most frequent type of pituitary disorder and more common in adult. Some of tumors result in secretion too many hormones that regulate important function in the body and some can cause gland to produce lower levels of hormones. Most of pituitary tumor are benign (Myoclonic, 2018; Ines *et al.*, 2014).

Any pituitary tumor give rise clinical features due to the distraction of normal pituitary tissue and of intracranial space occupying lesion. Lead to headache, vomiting and papilledema **(Marshall *et al*, 2012).**

2.2.4.2 Hypopituitarism

Hypopituitarism is defined as diminished function of pituitary gland. It also known as Simmonds's disease, there are two main reasons for hypo function of pituitary gland, it can be result from pituitary dysfunction (primary hypopituitarism) or from hypothalamic damage (secondary hypopituitarism). In both cases the production of pituitary hormones is decreased (Seong, 2015; Mareike *et al*., 2017).

Various causes of hypopituitarism in adult are: a pituitary adenoma and it is treatment by surgery or radiotherapy. Macro adenomas (greater than 1cm) are associated with one or more trophic hormones deficient in 30% of cases. While micro adenoma (less than 1 cm) rarely affect pituitary function, prolactin producing micro adenomas often present with hypogonadism because of the suppressive action of high prolactin level on gonadotropin (FSH and LH) secretion (Prabhakar and Shalet, 2006).

2.3 Testes

1.3.1 Definition

The testes are paired ovoid organs located outside of the body, in cased by muscular sac called scrotum which regulate the temperature of the testicular to 2°C below core body temperature. This important function is vital to uninterrupted sperm production (Bishop *et al*., 2010).

2.3.2 Anatomy

The testis is divided by septa of connective tissue in to about 250-300 tubules which called seminiferous tubules, that located in the walls of which the spermatozoa are formed from the primitive germ cells (spermatogenesis). Both

ends of each loop drain in to a network of ducts in the head of the epididymis from there spermatozoa pass through the tail of epididymis in to the vas deference.

They enter through the ejaculatory ducts in to the urethra in the body of the prostate at the time of ejaculation. Testis also contains other type of cells such as Sertoli cell and Leydig cells.

Sertoli cell, it is a cell that surround all stages of developing sperm, it also secretes signaling molecules that promote sperm production and can control either germ cell live or die. Leydig cells a type of interstitial cell located between the seminiferous tubules that produce testosterone (Parrett, 2010).

2.3.3 Function

The testes produce the male gametes and male sexual hormones, the term spermatogenesis describe and include all the processes involved in the production of gametes, whereas steroidogenesis refers to the enzymatic reaction leading to production of male steroid hormones, the function of the testes are governed by the hypothalamic and pituitary gland (endocrine regulation) these endocrine effects are mediated and modulated at the testicular level by local control mechanism (paracrine and autocrine factors) testes also produce inhibin B by sertoli cell and INSL3 by interstitial cell (Gerhard *et al*., 2010).

2.3.4 Clinical disorder

Testes disorders can be divided into:

2.3.4.1 Physical injury

The testes lie outside of body and are not protected by muscle or bone so any physical shock or trauma can cause severe pain, bruising and swelling this not serious but can lead to testicular rupture.

2.3.4.2 Disease and condition that affect testes function

2.3.4.2.1 Male infertility

Refers to the absence or reduced sperm production or the production of sperm that not function normally, there can be many causes: genetic and life style factors (Society for Endocrinology, 2015).

2.3.4.2.2 Sub fertility

Generally, describes any form of reduced fertility or poor semen quality. The most common causes of this condition include tubal disease, peritoneal adhesions and sperm related deficiencies; concerning the concentration, motility and morphology of spermatozoa (Gnoth *et al*., 2005; Adamson and Baker, 2003).

2.3.4.2.3 Hypogonadism

Male hypogonadism is the result of deficiency of the male sex hormone testosterone, leading to loss of sex drive and function, delayed puberty, osteoporosis and failure of testes to produce sperm, according to that it can be divided into:

1-hypogonadotrophic hypogonadism: is the occurrence of low testosterone levels together with low inappropriately normal FSH or LH levels. This condition associated with Kallmann syndrome, hyperprolactinemia, pituitary disease and also decline inversely with age after thirty years old.

2-HypergonadotropicHypogonadism, incorporate a group of disorder characterized by low testosterone, elevated FSH and LH, and impaired sperm production, this condition is associated with Klinefelter's syndrome, testicular feminization syndrome, Sertoli cell-only syndrome, testicular injury and infection (Bishop *et al.*, 2010).

2.4 Hypothalamic-pituitary-gonadal axis

Testosterone synthesis and male fertility are the result of perfect coordination of the hypothalamic-pituitary gonadal axis, impeccable coordination of the HPG axis is required for normal testicular function in the male, including normal testosterone production and male fertility. Pulsatile secretion of gonadotropin-releasing hormone (GnRH) by the hypothalamus travels down the anterior portion of the pituitary via the hypophyseal portal system and bind receptors on the secretary cells of adenohypophysis stimulates the biosynthesis of pituitary gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), LH triggers Leydig cells, which lie in the interstitial tissue of the testes between the seminiferous tubules. Stimulates the production of testosterone, which in turn, inhibits LH secretion by negative feedback**.** FSH triggers Sertoli cells, part of the basement membrane of the seminiferous tubules, The Sertoli cells are involved in germ cell differentiation and spermatogenesis. These functions depend on testosterone and are stimulated by FSH.

Inhibin is a hormone produced by the Sertoli cells which controls FSH secretion by negative feedback and activin enhances spermatogenesis (Corradi *et al*., 2016; Charlton, 2008; Martin, 2012).

2.5. Testosterone

2.5.1 Definition and physiological function:

Testosterone is the principal androgenic anabolic steroid in human. It is produced primarily in the testis of male (Oscar *et al*., 2010). Small amount is also produced by the ovaries of female and, the adrenal cortex in both male and female. Testosterone is converted to two other important hormones. It is reduced to dihydrotestosterone in specific tissues like the skin and the prostate and it is oxidized to estradiol. In men this oxidation mainly takes place in adipose tissue

and in the testes. Testosterone and dihydrotestosterone together are responsible for the typically male sex characteristics, but their function is different. In the adolescence testosterone induces the sex drive in men, enlargement of the penis, the production of sperm, increase of muscle mass and lowering of the voice, the so called anabolic effects. Dihydrotestosterone is responsible for an increase of body hair, beard grow, acne, and at a later age for baldness and enlargement of the prostate. These are the androgenic effects (Aede and Willem, 2007).

2.5.2. Biosynthesis and biochemistry

Testicular testosterone secretion is principally regulated by luteinizing hormone (LH) through its regulation of the rate-limiting conversion of cholesterol to pregnenolone within Leydig cell mitochondria by the cytochrome P-450 SCC enzyme located on the inner mitochondrial membrane. Cholesterol supply to mitochondrial steroidogenic enzymes is regulated by steroidogenic acute regulatory protein (stAR) which stimulates the flow of cholesterol from the outer mitochondrial membrane.to the inner mitochondrial membrane. All subsequent enzymatic steps are located in the Leydig cell smooth endoplasmic reticulum (Eacker *et al*., 2008).

The cholesterol is predominantly formed by de novo synthesis from acetyl-CoA, although preformed cholesterol either from intracellular cholesterol ester stores or extracellular supply from circulating low-density lipoproteins also contributes (Miller and Auchus,2011).

So the biosynthesis of the testosterone hormone starts with the oxidation of the side chain of cholesterol, which is catalyzed by the enzyme cytochrome $P450_{scc.}$

This cytochrome P450 oxidizes the side chain on C20 and C22 by the introduction of two hydroxyl groups. After that the chain is broken in between these two atoms by the same enzyme, under formation of pregnenolone. The next steps in the

biosynthesis of testosterone can proceed via two different routes. Pregnenolone can be oxidized first by cytochrome P450$_{17\,\Delta}$ to 17 Δ -hydroxy pregnenolone. This route is known as the 5-ene route because all biosynthetic intermediates in this route possess a Δ $_5$-double bond. The enzyme 3β-Hydroxysteroid dehydrogenase/Δ$_5$- isomerase (3β-HSD) also can convert pregnenolone first into progesterone by oxidation of the 3 Δ -hydroxy group followed by a shift of the double bond from the C5-C6 to the C4-C5 position. The enzyme 3 Δ -HSD first oxidizes the hydroxyl group at C3 to a carbonyl group and after that the same enzyme catalyzes the isomerization of the double bond to the Δ $_4$-position. This oxidation-isomerization reaction proceeds in only one direction. In principle this enzyme accepts all compounds in the left column of Scheme 1 as a substrate but pregnenolone and DHEA are the main substrates. This is indicated in Scheme 1 with bold arrows. This route is known as the 4-ene route because here all biosynthetic intermediates possess a Δ $_4$-double bond. Pregnenolone serves as a common substrate for the synthesis of Testosterone through the Δ5 or Δ4 pathways in the endoplasmic reticulum of Leydig cells. Despite the fact that the two pathways run in parallel and entail the same number of enzymatic reactions, most testosterone biosynthesis in the human testis takes place through the conversion of pregnenolone to dehydroepiandrosterone via the Δ5 pathway, because of a higher affinity of the steroidogenic enzymes involved for the metabolites of the Δ5pathway. Both pregnenolone and progesterone are accepted as substrate by the enzyme cytochrome P450$_{17\,\Delta}$. In both compounds ahydroxyl group is introduced in the 17 Δ -position, as is indicated in the name of the enzyme. The rupture of the C17-C20 bond is catalyzed by the same enzyme. This results in the complete removal of the side chain under formation of a carbonyl group at C17. In this way 4-androstene-3,17-dione (A-Dione) is obtained via the 4-ene route, and 3 Δ -hydroxy-5-androstene-17-one (DHEA) via de 5-ene route. The

acronym DHEA originates from the old-fashioned name dehydroepiandrosterone for this compound. DHEA has a weak anabolic activity from itself. The transformation of A-Dione into testosterone now only needs the reduction of the C17 carbonyl group to a 17-h Δ hydroxyl group. This reaction is catalyzed again by a dehydrogenase enzyme, called 17 Δ- hydroxy-steroid dehydrogenase or 17Δ-hydroxysteroid oxidoreductase or simply 17Δ- HSD. This enzyme adds two H-atoms to the carbonyl group, one to the O-atom and the other one to the bottom of C17, which results in the position of the hydroxyl group (Aede and Willem, 2007*).*

2.5.3. Metabolism

The metabolism of testosterone and dihydrotestosterone takes place for 90% in the liver (Owing to the presence of steroid catabolic enzyme). Finally, testosterone undergoes inactivation by hepatic phase I and II metabolism to inactive oxidized and conjugated metabolites for urinary and/or biliary excretion (David, 2016). Testosterone is converted to the most potent natural androgen DHT by the 5areductase enzyme that originates from two distinct genes (I and II). Type 1 5a--reductase is expressed in the liver, kidney, skin, and brain, whereas type 2 5areductase is characteristically expressed strongly in the prostate but also at lower levels in the skin (hair follicles) and liver .The inactivation mechanism include the following: addition of two hydrogens (reduction) to a double bond or ketone group; removal of two hydrogens (oxidation) from a hydroxyl group; addition of hydroxyl group (hydroxylation) to a carbon in the steroid molecule; and conjugation of steroids by reaction of sulfuric acid or glucuronic acid with a hydroxyl group on the steroid molecule, forming steroid sulfates and glucuronides, respectively Both groups enhance the polarity of the whole molecule considerably and in this way the a polar steroids become soluble in water and can be excreted with the urine (frank, 2009).

2.5.4. Testosterone secretion and transport

Testosterone is released in the general circulation via the spermatic vein in a pulsatile way. In young males, this occurs in a circadian manner, with a Testosterone peak observed in the early morning. Aging is associated with progressive loss of circadian Testosterone secretion (alexander, 2017).

Once synthesized, the lipophilic androgens move out of the Leydig cells by passive diffusion, down the concentration gradient. Within the testis, testosterone and precursors diffuse freely into the interstitial space and enter the testicular blood capillaries that are immediately adjacent to Leydig cells (Stephen and Winters, 2017).

Interestingly, it is this process of testosterone release into the testicular vascular bed which might be altered in Klinefelter syndrome leading to reduced circulating testosterone levels. Once they are part of the systemic circulation, secreted testosterone binds to plasma proteins and is present in both bound and unboundforms. In adult humans, more than 95% of testosterone is complexed with proteins, both the high affinity sex hormone binding globulin (SHBG) and the low affinity albumin. The proportion of testosterone that is unbound or loosely bound represents the biologically active fraction, which freely diffuses from capillaries into cells. The SHBG-bound fraction is thought to act as a reservoir for the steroid, although SHBG bound steroids may also enter cells via endocytic receptors on the surface of target cells and contribute to hormone action. Increasing levels of SHBG during aging contributes to reduced free plasma testosterone during this period (Tuttelmann,2014).

2.5.5. Pathophysiology

In general, a normal male testosterone level peaks at about age 20, and then it slowly declines. Sometimes significant changes in testosterone levels occur and is it termed hypogonadism, "male menopause" or andropause (Nayana, 2017).

2.5.5.1. Some causes of low testosterone levels

Some causes of low testosterone level are age**,** injury, Chemotherapy or radiation treatment, Klinefelter's Syndrome, Dysfunction of the pituitary gland, corticosteroid drugs, chronic kidney failure, Stress. Alcoholism and smoking, Obesity (especially abdominal) and Kallmann syndrome lead to decrease in testosterone production (Nayana, 2017).

2.5.5.1.1. physical changes Caused by Low testosterone

Low testosterone can cause the following physical changes: Infertility, Decrease in muscle mass with an increase in body fat, Changes in cholesterol levels, mild anemia, Fragile bones, Decrease in body hair (Nayana, 2017).

2.5.6 Diagnosis of low testosterone:

Measure the amount of testosterone in your blood. Because testosterone levels fluctuate throughout the day, several measurements will need to be taken to detect a deficiency. Doctors prefer, if possible, to test levels early in the morning, when testosterone levels are highest (Nayana, 2017).

2.5.7. Treatment of low testosterone:

Testosterone deficiency can be treated by: Intramuscular injections, given anywhere from two to 10 weeks apart, Testosterone gel applied to the skin or inside the nose, Mucoadhesive material applied above the teeth twice a day, Long-acting subcutaneous pellet, Testosterone stick (apply like underarm deodorant (Nayana, 2017).

2.6 Prolactin:

Prolactin is a polypeptide hormone that is synthestized in and secreted from specialized cells of the anterior pituitary gland, the lactotrophs. The hormone was originally named for its ability to promote lactation in response to the suckling stimulus of hungry young mammals(Freeman, Kanyicska, Lerant, & Nagy, 2000).

2.6.1 Function

The main biological action of prolactin is inducing and maintaining lactation. However, it also exerts metabolic effects, takes part in reproductive mammary development and stimulates immune responsiveness. All these effects of prolactin are because it binds to specific receptors in the gonads, lymphoid cells, and liver (Voicu, Medvedovici, & Ranetti, 2013).

Prolactin is also involved in osmo regulation, increasing water and salt absorption in all segments of the bowel and reducing renal Na+ and K+ excretion (Capozzi, Scambia, Pontecorvi, & Lello, 2015)

2.6.2 Structure

Prolactin (PRL) is a 23 kDa single-chain polypeptide of 199 amino acids, with three intramolecular disulphide bonds between six cysteine residues (Cys4-Cys11, Cys58-Cys174, Cys191-Cys199) (Syndr, Maria, & Gene, 2016) **.** Approximately 80 -- 90% of the circulant prolactin is monomeric, but dimeric or polymeric forms, with high molecular weights, could also be observed(Holt & Holt, 2008).

2.6.3 Prolactin receptors (PRLR)

Prolactin receptor (PRL-R) isa trans membrane protein of the cytokine/hematopoetin receptor superfamily, structurally related to the GH, the granulocyte-macrophage colony stimulating factor (GM-CSF), the erythropoietin and the interleukin (IL)-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-13 and IL-15 receptors(Capozzi et al., 2015).

The gene that encodes the human PRLR is found in chromosome 5 and contains at least 10 exons. The transcriptional function of the gene PRLR is achieved by 3 different promoters: Promoter 1 special for gonads; Promoter 2 for Liver; Promoter 3 for all tissues(Syndr et al., 2016).

The prolactin receptors are produced in various types of cells, with several isoforms and transported at the level of the cell membrane. The life cycle of the receptor ends by its removal from the plasma membrane(Voicu et al., 2013)

2.6.4 Prolactin secretion

Prolactin secretion proceeds via combined pulsatile (burst-like)and basal (time-invariant) modes of release. A complicating issue in defining normative ranges even for measures of PRLsecretion is that they may depend upon one or more biological or clinical factors, such as gender, age, BMI, sex-steroid concentrations, core temperature, nutrition, stress, exercise, medications and renal disease. Prolactin secretion is primarily regulated by the inhibitory action of hypothalamic dopamine, and by ultrashort auto feedback, but the physiological role of various releasing hormones is not established in man. These factors would putatively determine more complex PRL dynamics, which arise physiologically from feed forward (stimulatory) and feedback (inhibitory) signals interacting in an integrative fashion, as demonstrated in detail for GH and LH in men. Novel integrative measures are approximate entropy (ApEn) and spikiness, which reflect the complexity and stability of signaling interactions in homeostatic systems. (Roelfsema, Pijl, Keenan, & Veldhuis, 2012).

2.6.5 Prolactin Clearance

The metabolic clearance of prolactin (PRL) is partially executed by the kidney PRL has a longer half-life and a lower metabolic clearance rate in the circulation of patients with CKD . The latter may be due to impaired renal degradation, which is significant in the healthy kidney as the PRL concentration in the renal vein is approximately 16% lower than in the renal artery . In CKD, the arteriovenous PRL concentration gradient may be lower due to a decline of functional kidney tissue, which could favor the rise in circulating PRL levels reported in these patients . This possibility is also supported by the observation that after renal transplantation,

or upon resolution of CKD for other reasons, PRL levels usually return to normal (Triebel et al., 2015).

2.6.6 Clinical application

The term ‘‘hyperprolactinemia’’ refers to an increase in circulating PRL levels, usually producing reproductive problems in both sexes particularly anovulatory infertility in women (Torre, 2007)

Hyperprolactinemia is classified in two types: the functional or organic hyperprolactinemia. The most common reasons of functional mild to moderate hyperprolactinemia are a variety of pharmacotherapeutic agents that reduce hypothalamic secretion of dopamine or its action in the pituitary(Madhusoodanan, Parida, & Jimenez, 2010).

Functional hyperprolactinemia is typically observed in pregnancy but it often occurs in polycystic ovary syndrome (PCOS), renal failure, hepatic cirrhosis anvbnmd renal and lung cancers (Robin et al., 2011). Pathological functional hyperprolactinemia also recurs in endocrinopathies such as primary hypothyroidism and primary adrenocortical insufficiency (Cortet-Rudelli et al., 2007). Mild stress, including that of venipuncture, can induce transient elevations in serum PRL(Ignacak et al., 2012)

Chapter Three

Material and methods

Chapter Three

Materials and Methods

3.1 Materials

3.1.1 Study Design

This study is analytical case - control study.

3.1.2 Study population

This study was conducted in Alturkey hospital and ultra lab in Khartoum state on 40 subjects with CKD as case with stage 5 and 40 subjects from healthy individuals as control age ranged from 26 to 81 years.

3.1.3 Study Subjects

3.1.3.1 Inclusion criteria:

Patients clinically diagnosed with CKD were included stage 5 dialyzed in Alturkey hospital .

3.1.3.2 Exclusion criteria

Patients with CKD who are either smoker, hypothyrodism, alcoholism, pituitary tumor or high dose or long term use of steroid were excluded from this study.

3.1.4 Sampling technique

The study sample was selected by convenience technique.

3.1.5 Data collection

The Data was collected from all study groups by using questionnaire.

3.1.6 Blood sampling:

Venous blood was collected before dialyzing into appropriately labeled tube and allows it to clot. Centrifuge at 1300-2000 rpm for 10 minutes and carefully remove the serum layer. Stored at -20 until analyzed. Consider all human specimens as possible bio hazardous materials and tack appropriate precautions when handling. Approximately 0.3 ml of serum is required.

3.1.7 Ethical consideration

This study ethically provided by Sudan University of Science and technology ethical clearance committee, and then informed consent was taken from all participants.

3.2 Methods

3.2.1 Prolactin

3.2.1.1 Principle of the method (appendix 1)

The assay system utilizes as polyclonal anti-prolactin antibody for solid phase (Microplate wells) immobilization and mouse monoclonal anti-prolactin antibody in the antibody enzyme conjugate solution. The test sample is allowed to react simultaneously with antibodies, resulting in prolactin molecule being sandwiched between the solid phase and enzyme-linked antibodies.

After 60 min incubation at room temperature, the wells were washed to remove unbound labeled antibodies a solution substrate was added and incubated for 20 min, resulting in the development of blue color. The color development is stopped with the addition of 2N HCL, and the color was changed to yellow and measured 33 spectrophotometrically at 450nm.

The concentration of prolactin was directly proportional to color intensity of test sample.

3.2.2 Testosterone

3.2.2.1 Principle of the method (appendix 2)

ST AIA-BACK testosterone was competitive enzyme immunoassy which was performed entierly in the STAIA-BACK testosterone test cups. testosterone present in the test sample compete with enzyme lable testosterone for limited number of binding site on testosterone specific monoclonal antibody immoblized on magnetic solid phase. The magnetic beads were washed to remove un bound enzyme labled testosterone and then incubated with afluorogenic substrate 4-methyl lumblliferyl phosphate (4MUP). The amount of enzyme labeled testosterone that binds to the beads was inversely propositional to testosterone concentration is the test sample

Standard curve was constructed and unknown sample concentration are calculated using this curve.

3.2.3 Quality control:

A daily maintenance was firstly done then a set of two levels of human assayed control made by had been analyzed with each batch of samples. In addition, when the control do not recover within the acceptable tolerances range, corrective actions such as a new calibration and specific maintenance or troubleshooting procedures were done.

3.2.4 Statistical analysis:

Data have been analyzed using SPSS software program version 21. Results were expressed as mean ± SD. Independent T test were used to compare mean concentration of hormones in case and control and to compare the mean concentration of parameters with variable and Personal correlation test used to correlate between hormones concentration and continuous variable. P-Value less than 0.05 were regarded as significant.

Chapter Four

Results

Chapter Four

Results

4-1 Base line characteristics of patients

Table (4-1) the frequency analysis to diabetes showed that, 10(25%) are diabetic and 30 (70%) non diabetic.

Frequency analysis to hypertension showed that, 27(67%) are hypertensive and 13(33%) non hypertensive.

Frequency analysis to medication showed that, 17(43%) take medication and 23(57%) do not take medication.

4.2 Distribution of patients according to BMI classification

Table (4-2) the frequency analysis to BMI showed that, 12(30%) are under weight, 24(60%) normal weight, 3(7.5%) over weight, 1(2.5%) obese.

4.3 Mean of study variable among study population

Table (4-3) show mean of study variable, the range of age from 26to 81 years with mean 52.5±14.7, the range of BMI from 13.7to 35.5 kg/m2 with mean 21.1±4.78, and range of duration from 1 to 15 years with mean 4.80±3.39.

4.4 Comparison of PRL and testosterone in case versus control group

Table (4-4) shows that prolactin was significantly increased in CKD patients comparing with control group (p. value 0.000) while testosterone was significantly decreased in CKD patients comparing with control group (p.value0.000), result expressed as (mean ± SD).

4.5 **Comparison of PRL and testosterone across DM**

Table (4-5) show there was significant increase in prolactin in diabetic patients compared to non-diabetic patients (p-value 0.038), also significant decrease in testosterone in diabetic patients compared to non-diabetic patients (p-value 0.033).

4.6 Comparison of PRL and testosterone across hypertension

Table (4.6) showed that there was insignificant difference between hypertensive& non hypertensive in the level of prolactin and testosterone in chronic kidney disease patients.

4.7 comparisons of PRL and testosterone across medication

Table (4.7) showed that there was insignificant difference between patients had taken medication and no taken medication in the level of prolactin and testosterone in chronic kidney disease patients.

4.8 correlations between testosterone level and age

Figure (4-1) showed that testosterone level was negatively correlated with age (R= -0.562**, P=0.000). This indicates increasing in age will lead to decrease in testosterone levels**.**

4.9 correlations between PRL level and age

Figure (4-2) showed was no correlation between PRL and age (R =0.158, P =0.333)

4.10 correlations between testosterone level and duration

Figure (4-3) showed that there was no correlation between testosterone and duration (R= -0.064, P=0.694).

4.11 correlations between PRL level and duration

Figure (4-4) showed PRL level was positively correlated with duration (R= 0.726**, p= 0.000).this indicate increasing in duration will lead to increase in PRL levels.

4.12 correlations between testosterone and BMI

Figure (4.5) showed there was no correlation between testosterone and BMI (R =-0.088, P = 0.588).

4.13 Correlation between PRL and BMI

In figure (4.6) showed PR Level was positively correlated with BMI(R =0.639**, P =0.000).this indicate increasing in BMI lead to increase in PRL level.

Table (4-1) Baseline characteristics of patients

Variables	Frequency	Percentage (%)
DM		
Yes	10	25.0
No	30	75.0
H.T		
Yes	27	67.0
No	13	33.0
Medication		
Yes	17	43.0
No	23	57.0
Total	**40**	**100.0**

Table (4-2) Distribution of patients according to BMI classification

Variable	Frequency	Percentage (%)
Under weight	12	30.0
Normal weight	24	60.0
Over weight	3	7.5
Obese	1	2.5
Total	**40**	**100.0**

Table (4-3) Mean of age, BMI and duration in case group

Variables	Minimum	Maximum	Mean±SD
Age	26.0	81.0	52.5±14.7
BMI	13.7	35.5	21.1±4.78
Duration	1.00	15.0	4.80±3.39

Table (4-4) Mean comparison of PRL and testosterone in case versus control group

Parameters	Case (Mean±SD)	Control (Mean±SD)	*P-value*
PRL (ng/ml)	14.07±7.51	7.00±2.69	0.000
Testosterone (ng/dI)	412.25±152.58	684.48±184.96	0.000

Table (4-5) Mean comparison of PRL and testosterone across DM

Parameters	Yes (Mean±SD)	No (Mean±SD)	*P-value*
PRL (ng/ml)	15.88±8.35	13.46±7.26	0.038
Testosterone (ng/dI)	339.2±100.5	436.6±160.4	0.033

Table (4-6) Mean comparison of PRL and testosterone across H.T

Parameters	Yes (Mean±SD)	No (Mean±SD)	*P-value*
PRL (ng/ml)	14.62±8.43	12.92±5.22	0.440
Testosterone (ng/dI)	421.3±103.9	393.4±97.8	0.066

Table (4-7) Mean comparison of PRL and testosterone across medication

Parameters	Yes (Mean±SD)	No (Mean±SD)	*P-value*
PRL (ng/ml)	15.62±7.77	12.92±7.28	0.267
Testosterone (ng/dI)	398.6±132.2	422.3±168.2	0.620

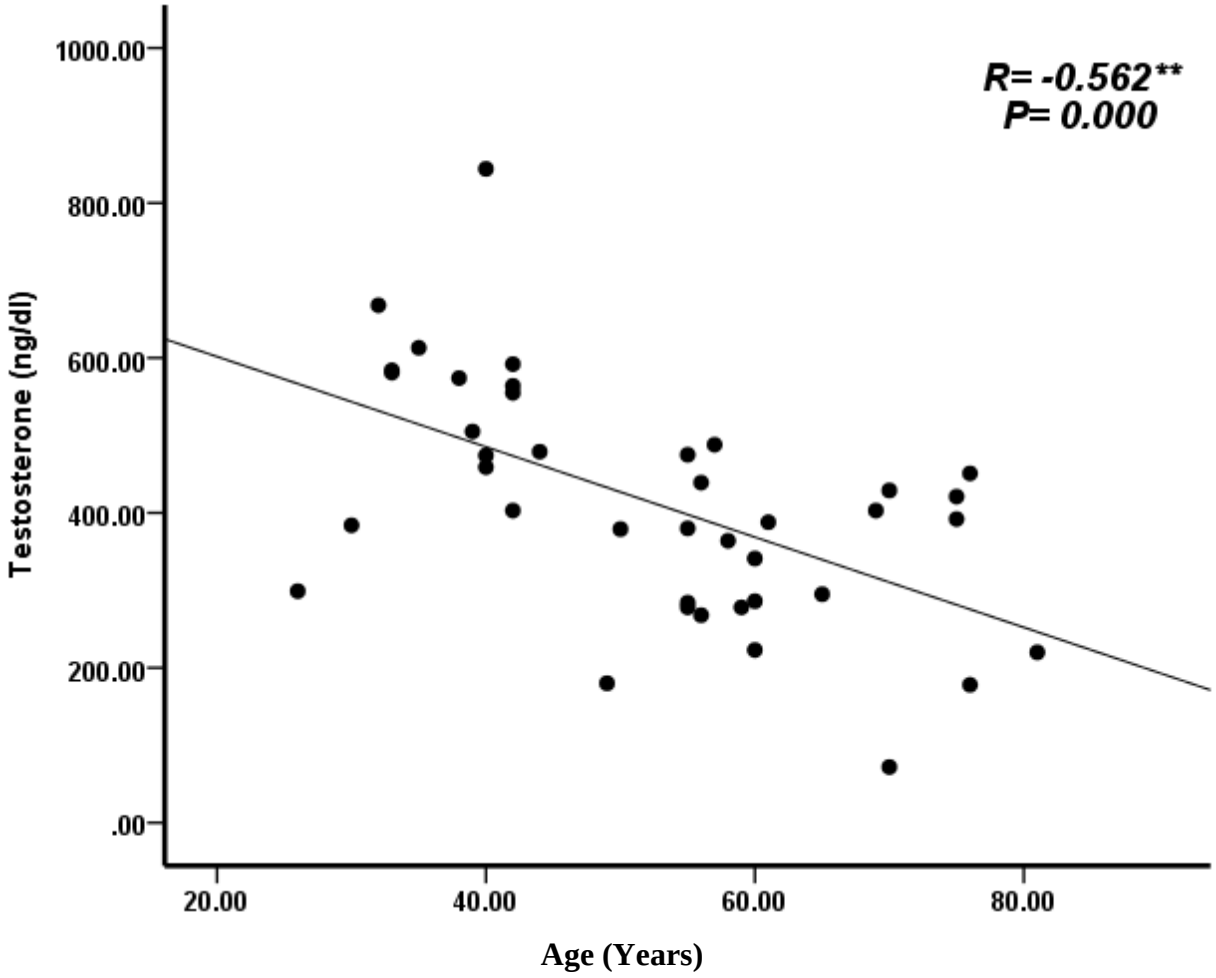

Figure (4.1) correlation between testosterone level and age(R = ˉ0.562, P = 0.000)**

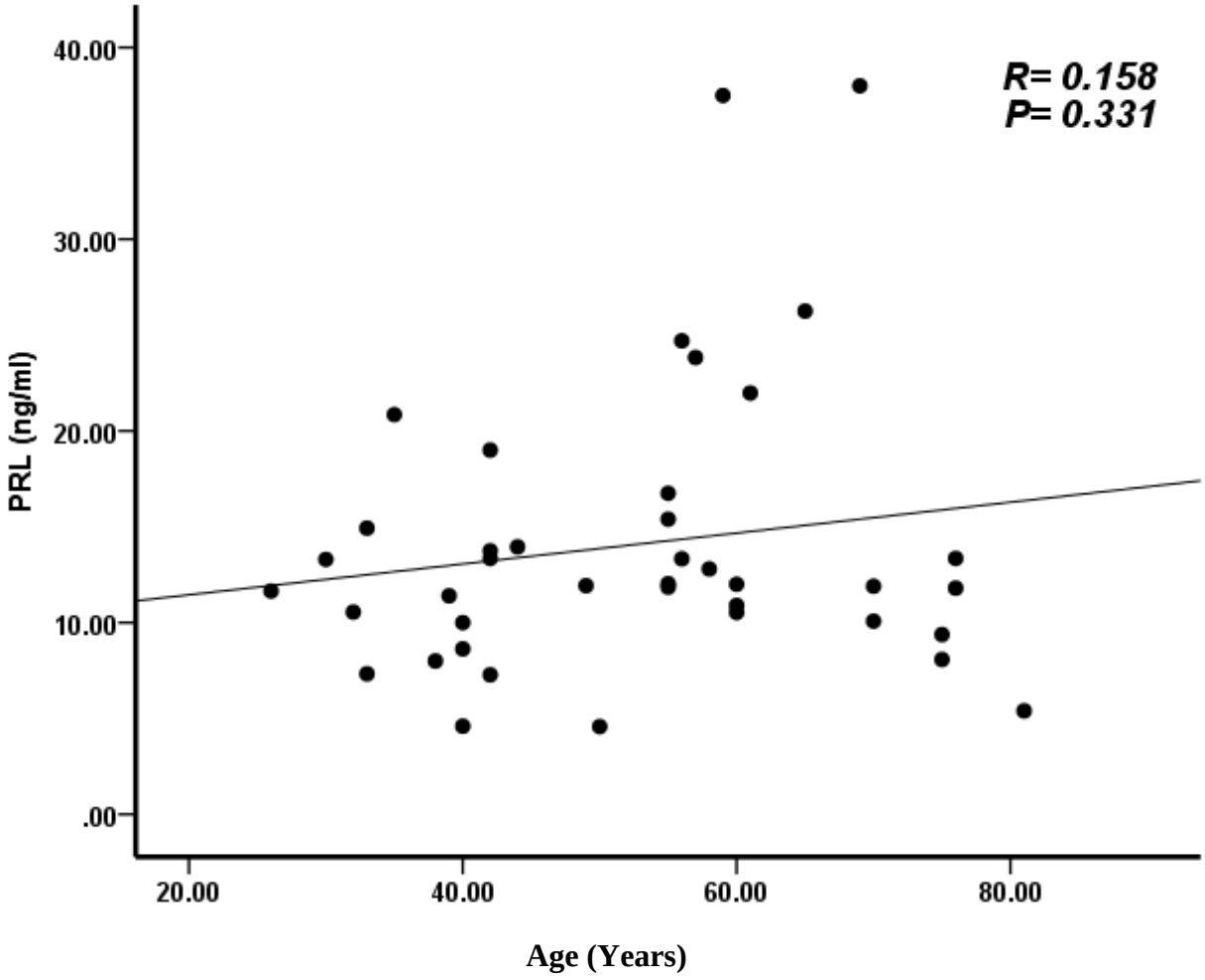

Figure (4.2) correlation between PRL level and age(R = 0.158, P = 0.331)

-

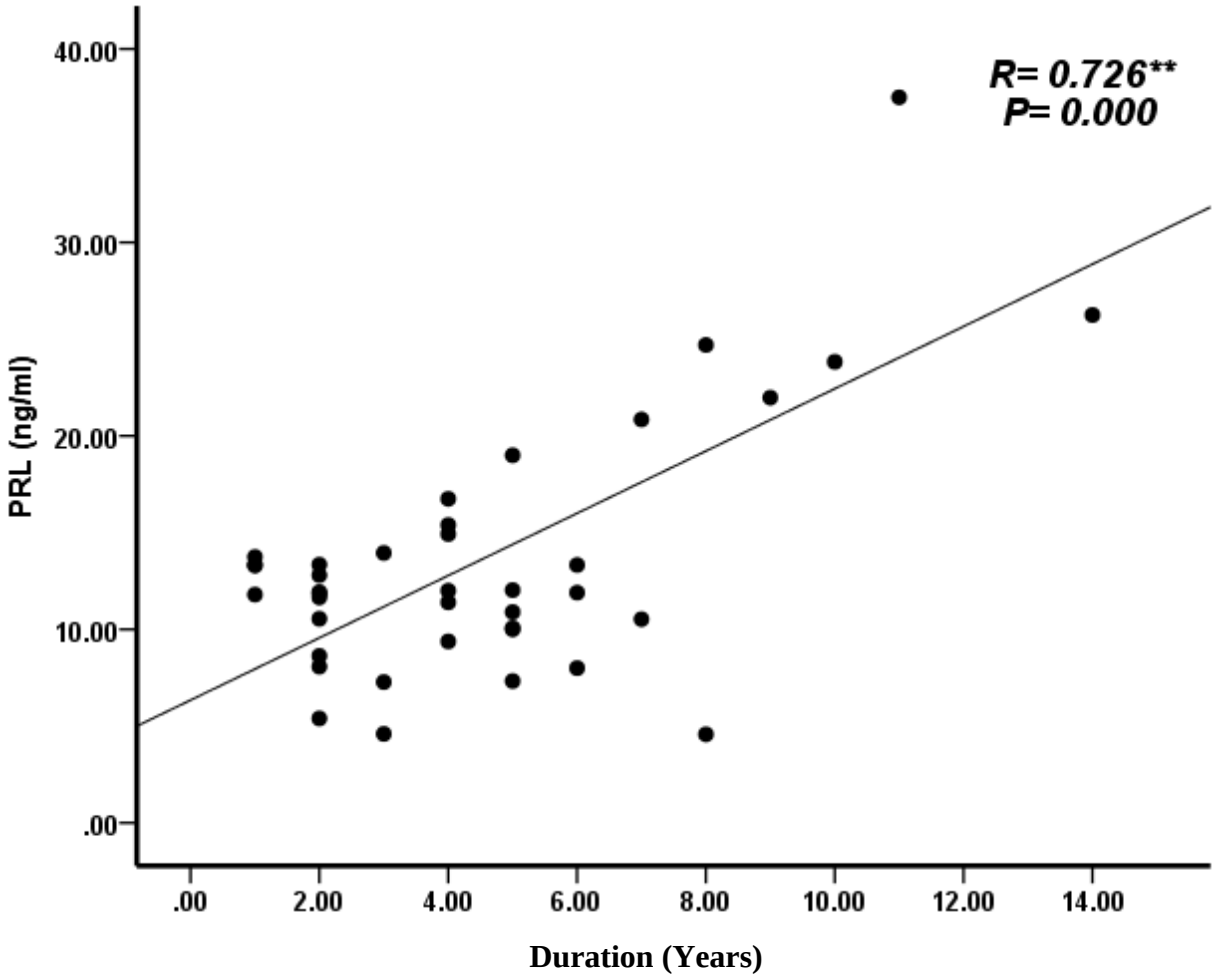

Figure (4.3) correlation between PRL level and duration(R = 0.762, P = 0.000)**

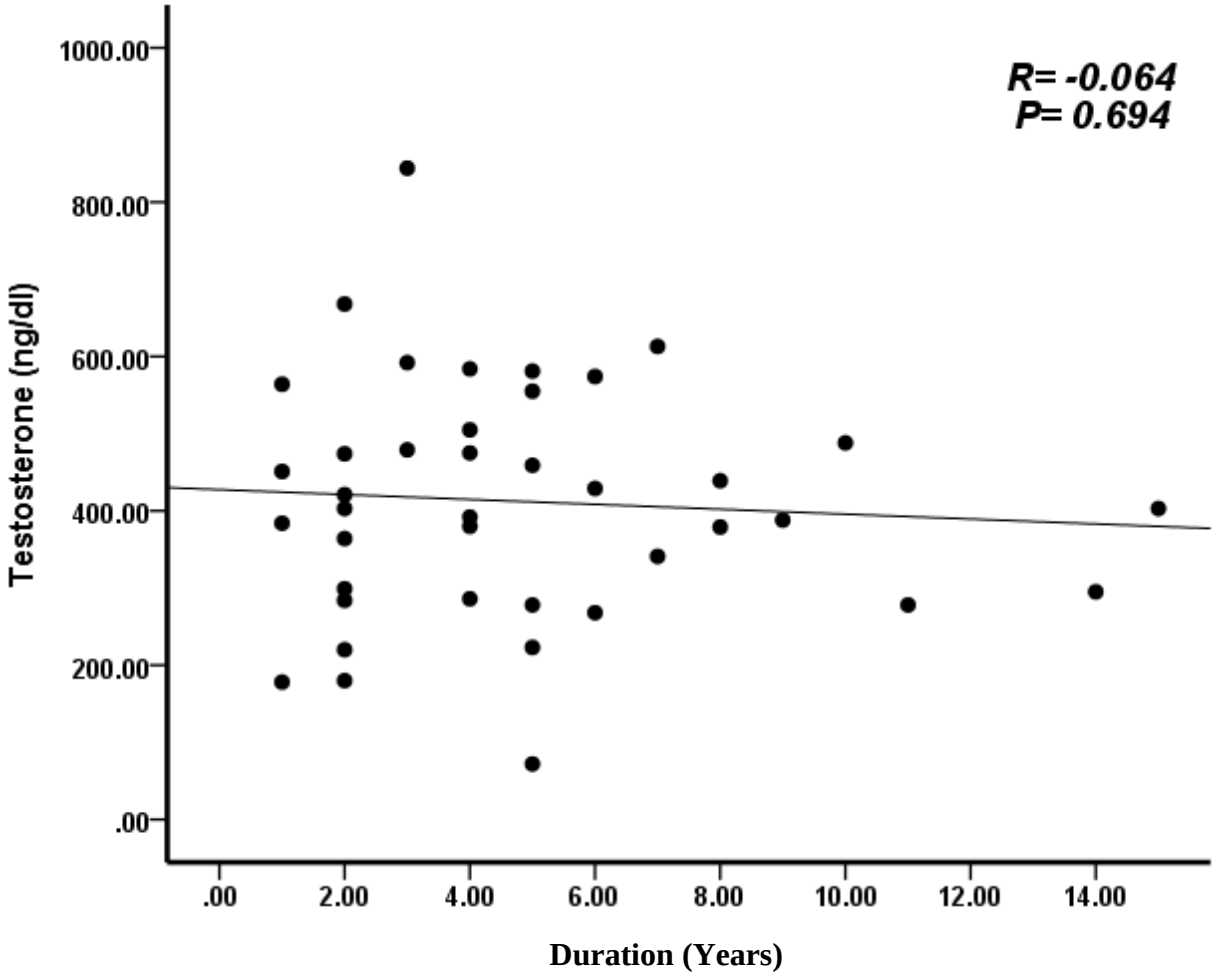

Figure (4.4) correlation between Testosterone level and duration (R = -0.064, P = 0.694)

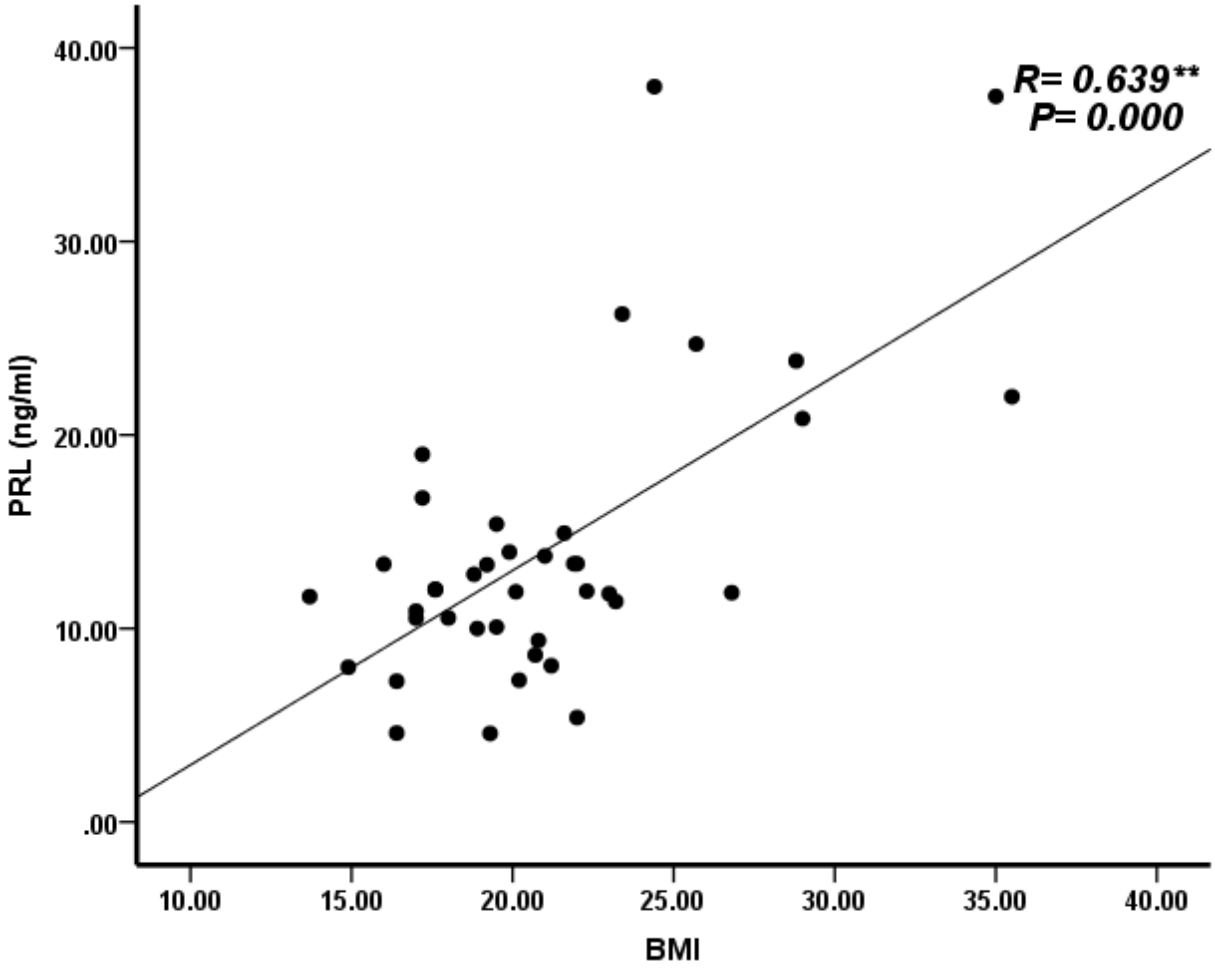

Figure (4.5) correlation between PRL level and BMI(R = 0.639, P = 0.000)**

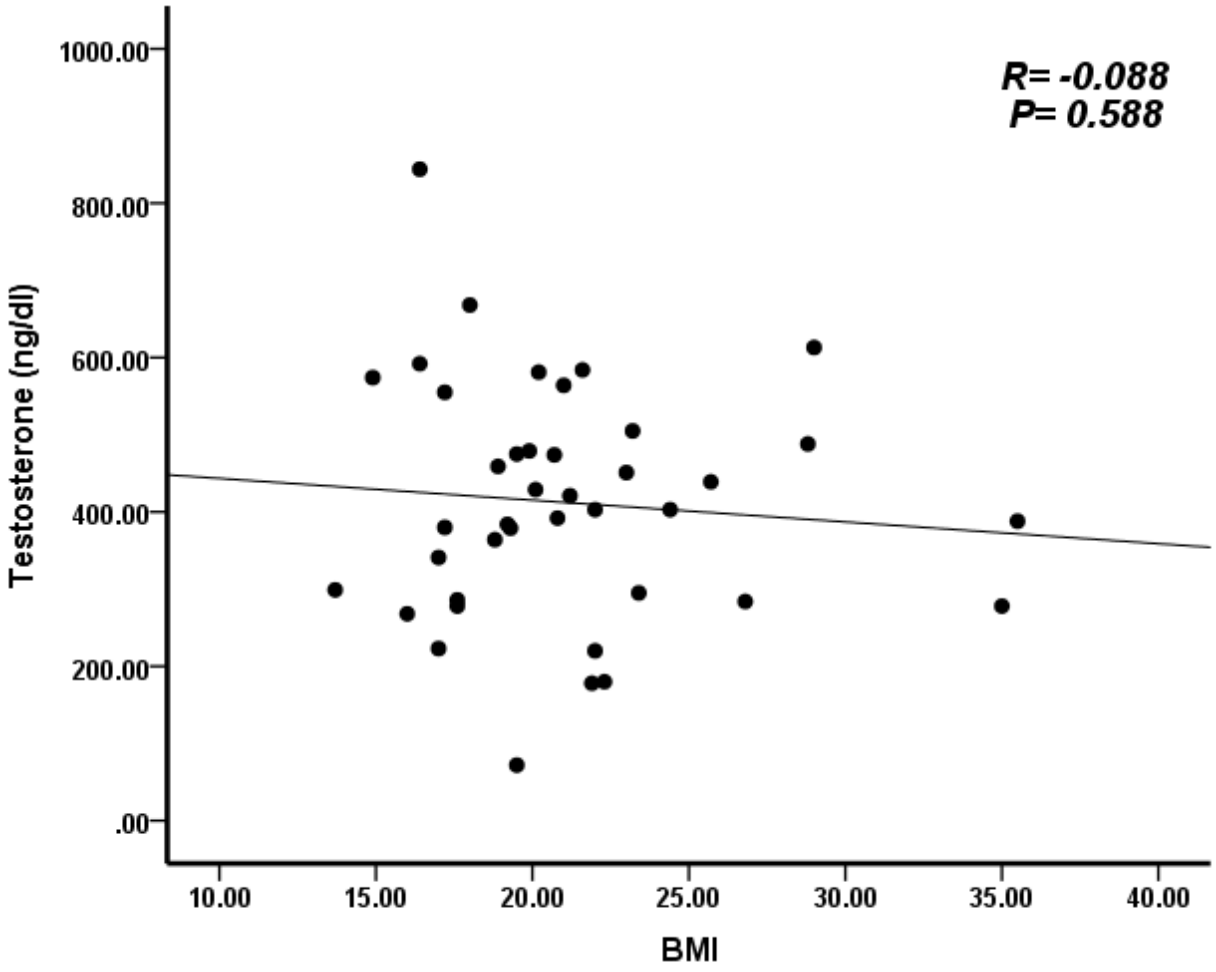

Figure (4.6) correlation between Testosterone level and BMI(R = -0.088, P = 0.588)

Chapter Five

Discussion

Chapter Five

Discussion, Conclusion and Recommendations

5.1 Discussion

This study was carried out to assess serum prolactin and testosterone among male chronic kidney disease patients. The results showed significant increased in prolactin in CKD patients compared with control group (*p*-value 0.00), our findings agreed with previous study stated that, hyperprolactinemia occurs commonly in CKD with a prevalence of 30–65% and this excess is due to a combination of diminished prolactin removal by the kidney and increased synthesis (Edey, 2017).

Concerned with previous reports suggested significant decreased in serum testosterone in CKD males, we found that, testosterone level decreased significantly in CKD patients compared with control group (*p*-value 0.00). In fact, uremia impairs gonadal steroidogenesis and lead to testicular damage. In addition, there was an inhibitory factors in uremic serum which inhibit LH signaling at the level of the Leydig cells (Priya and Rebecca, 2007; Mahboob and Shirin, 2008).Furthermore, high level of prolactin lead to loss of libido, gynecomastia, galactorrhea, infertility and secondary hypogonadismin male (Osman and Ismail, 2016).

Serum prolactin level increased significantly in diabetic patients compared with non-diabetic patients (*p*-value 0.038). Our results were in line with earlier study reported that, the circulating levels of PRL increased in diabetic patients and hyperprolactinemia lead to vaso-inhibin accumulation within the retina; genetic deletion of the PRL receptor prevented this effect, indicating receptor-mediated

incorporation of systemic PRL into the eye. Hyperprolactinemia reduced both VEGF-induced and diabetes-induced increase of RVP(Ahead, 2010)

Moreover, there was significant decrease in testosterone in diabetic patients compared to non-diabetic patients (*p*-value 0.033).accordingly, previous evidence reported low serum testosterone level and type 2 diabetes with insulin resistance and obesity as central feature (Kit et al, 2014).

The previous observations reported no significant variation between hypertensive& non hypertensive patients in levels of serum prolactin (Morgado et al, 2012) and testosterone(Kit et al, 2014). Our findings also revealed insignificant differences in prolactin and testosterone levels between hypertensive and non hypertensive CKD patients. Furthermore, there were insignificant differences in levels of prolactin and testosterone in patients taken medication compared with non-taken medication.

The correlation analysis revealed that, serum testosterone level negatively correlate with age (r: -0.562, *p*-value 0.000), while no correlation of testosterone with duration and BMI. These results confirmed with previous studies noted that, there were negative correlation between testosterone and age (Mastrogiacomo *et al*, 2009 and Hylander and Lehtihet, 2015) but no correlation of testosterone with duration (Park, Koo and Lee, 2013) and BMI (Osman and Ismail, 2016).

Serum prolactin level correlate positively with duration (r: 0.726, *p*-value 0.000) and BMI (r: 0.639, *p*-value 0.000), while no correlation between prolactin and age. These findings were in line with other previous reports showed positive correlation of PRL with duration(Kopelman and George, 2015) and BMI (Pereira-lima *et al*, 2013),while no correlation of PRL with age (Johnson *et al*,2013).

5.2 Conclusion

This study concludes that the level of testosterone hormone is decreased while prolactin is increased in CKD patients. also, there was significant increase in PRL and significant decrease in testosterone in diabetic patients had chronic kidney disease.

There was negative correlation between testosterone and age and no correlation between duration, BMI with testosterone. No correlation between PRL and age but positive correlation between BMI, duration with PRL.

5.3 Recommendation

1) Regular follow up of fertility hormones are recommended to avoid progression of sexual dysfunction and other disease related to hypogonadism such as bone disorders, CVD, anemia and low muscle mass.

2) Large sample size is needed to reach a strong conclusion regarding the effect of BMI on testosterone.

3) Subsequent studies may include other parameters (inhibin B, SHBG and hormone receptors) for more prediction regarding fertility status in HDP.

4) Subsequent studies should include fertility hormone in females with CKD.

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Sudan University of Science and Technology
Faculty of Medical Laboratory Sciences
Clinical Chemistry Department

QUESTIONNAIRE

No ( )

Date: / / 2018

Name: …….

Age: …….. Years.

Height …….. Weight ……. BMI ……

Duration of disease …….

Number of dialysis per week: …….

DM ☐ **Hypertension** ☐

Medications: ……

Results

Prolactin …… (ng/ml)
Testosterone: …….. (ng/dl)

Appendixes

Appendix (1)

BIOS

PROLACTIN

ENZYME IMMUNOASSAY TEST KIT
Catalog Number: 10006

Enzyme Immunoassay for the Quantitative Determination of Prolactin Concentration In Human Serum

(96 Tests)

Intended use

For the quantitative determination of prolactin concentration in human serum.

Introduction

Human prolactin (lactogenic hormone) is secreted from the anterior pituitary gland in both men and woman. Human prolactin is a single chain polypeptide hormone with a molecular weight of approximately 23,000 daltons. The release and synthesis of prolactin is under neuroendocrinal control, primarily through Prolactin Releasing Factor and Prolactin Inhibiting Factor. Women normally have slightly higher basal prolactin levels than men; apparently, there is an estrogen-related rise at puberty and a corresponding decrease at menopause. The primary functions of prolactin are to initiate breast development and to maintain lactation. Prolactin also suppresses gonadal function. During pregnancy, prolactin levels increase progressively to between 10 and 20 times normal values, declining to non-pregnant levels by 3-4 weeks post-partum. Breast-feeding mothers maintain high levels of prolactin, and it may take several months for serum concentrations to return to non-pregnant levels. The determination of prolactin concentration is helpful in diagnosing hypothalamic-pituitary disorders. Microadenomas (small pituitary tumors) may cause hyperprolactinemia, which is sometimes associated with male impotence. High prolactin levels are commonly associated with galactorrhea and amenorrhea. Prolactin concentrations have been shown to be increased by estrogens, thyrotropin-releasing hormone (TRH), and several drugs affecting dopaminergic mechanism. Prolactin levels are elevated in renal disease and hypothyroidism, and in some situations of stress, exercise, and hypoglycemia. Additionally, the release of prolactin is episodic and demonstrates diurnal variation. Mildly elevated prolactin concentrations should be evaluated taking these considerations into account. Prolactin concentrations may also be increased by drugs such as chloropromazine and reserpine, and may be lowered by bromocyptine and L-dopa.

Principle of the test

The Prolactin Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-prolactin antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-prolactin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the prolactin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 60 minute incubation at room temperature, the wells are washed to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 2N HCl, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of prolactin is directly proportional to the color intensity of the test sample.

Materials and components

Materials provided with the test kits:

- Antibody-coated microtiter wells
- Reference standard set, contains 0, 5, 20, 50, 100, and 200 Ng/ml human prolactin, in liquid form (ready to use) or lyophilized form.
- Enzyme Conjugate Reagent, 12 mL.
- TMB Substrate , 12 mL.
- Stop Solution , 12 mL.
- Wash Buffer Concentrate(50X), 15mL

Materials required but not provided:

- Precision pipettes: 40µL~ 200µL, and 1.0mL.
- Disposable pipette tips.
- Distilled water.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel
- Graph paper.
- Microtiter well reader.

Specimen collection and preparation

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

Storage of test kits and instrumentation

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

Reagent preparation

1. All reagent should be brought to room temperature (18-22°C) before use.
2. If reference standards are lyophilized, reconstitute each standard with 0.5 mL distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be sealed and stored at 2-8°C.
3. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 mL of Wash Buffer (50x) into distilled water to prepare 750 mL of washing buffer (1x). Mix well before use.

Assay procedures

1. Secure the desired number of coated wells in the holder. Make data sheet with sample identification.
2. Dispense 50µL of standard, specimens, and controls into appropriate wells.
3. Dispense 100 µL of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 10 seconds. It is very important to have complete mixing in this step.
5. Incubate at room temperature (18-22°C) for 60 minutes.
6. Remove the incubation mixture by flicking plate content into Sink.
7. Rinse and flick the microtiter wells 5 times with washing buffer(1X).
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100µL of TMB substrate into each well.Gently mix for 5 seconds.
10. Incubate at room temperature for 20 minutes.
11. Stop the reaction by adding 100 µL of Stop Solution to each well.
12. Gently mix for 5 seconds. It is important to make sure that All the blue color changes to yellow color completely.
13. Read optical density at 450nm with a microtiter well reader.

Important Note

The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

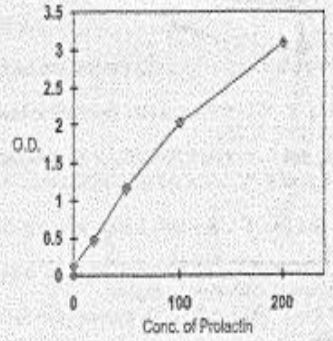
Calculation of results

Calculate the mean absorbance value (A_{450}) for each set of reference standards, specimens, controls and patient samples. Constructed a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/mL on graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of prolactin in ng/mL from the standard curve.

Example of standard curve

Results of typical standard run with optical density reading at 450nm shown in the Y axis against PRL concentrations shown in the X axis.

Prolactin (ng/mL)	Absorbance (450nm)
0	0.010
5	0.121
20	0.472
50	1.158
100	2.022
200	3.077



This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

Expected values and sensitivity

Each laboratory must establish its own normal ranges based on patient population. Based on a limited number of healthy adult blood specimens, the mean prolactin concentrations in males (N=90) and females (N=120) are estimated to be 6 and 15 ng/m L, respectively. The minimal detectable concentration of human prolactin by this assay is estimated to be 2 ng/mL.

Performance characteristics

1.Accuracy:Comparison between Chemux and commercial available Kits provide the following data
N = 120
Correlation Coefficient = 0.9860
Slope = 0.92
Intercept = 0.80
Mean (Chemux) = 17.41
Mean (Abbott) = 16.84
2.Precision
1].Intra-Assay:

Concentrations	Replicates	Mean	S.D.	% CV
Level I	20	7.21	0.371	5.10
Level I	20	17.25	0.850	4.90
Level II	20	41.73	1.998	4.80

2). Inter-Assay:

Concentrations	Replicates	Mean	S.D.	% CV
Level	20	7.43	0.740	10.0
Level I	20	18.11	1.337	7.40
Level II	20	42.65	2.26	5.30

3.Linearity
Two patient sera were serially diluted with 0 ng/mL standard in a linearity study. The average recovery was 100.5 %.

Sample A			
Dilution	Expected	Observed	% Recov.
Undiluted	179.3	179.3	
2x	89.7	95.4	106.4%
4x	44.8	42.9	95.75
8x	22.4	21.7	96.8%
16x	11.2	12.4	110.7%
Average Recovery: 102.4 %			

Sample B			
Dilution	Expected	Observed	% Recov.
Undiluted	201.6	201.6	
2x	100.8	104.6	103.8%
4x	50.4	52.3	103.8%
8x	25.2	23.3	92.5%
16x	12.6	11.9	94.4%
Average Recovery: 98.6 %			

4.Recovery
Various patient serum samples of known prolactin levels were mixed and assayed in duplicate. The average recovery was 101.4 %.

Expected Concentration	Observed Concentration	% Recovery
10.8	11.3	104
25.6	24.9	97.3
58.9	61.3	104.1
100.2	97.2	97.0
220.6	230.0	104.3
Average Recovery: 101.4 %		

5.Sensitivity
The minimum detectable concentration of this assay is estimated to be 2.0 ng/mL.
Cross-reactivity
The following human materials were tested for crossreactivity of the assay:

Antigens	Concentration	Equivalent Prolactin	%Cross-Reactivity
LH	500 mIU/mL	0.0 ng/mL	0.0
TSH	200 µIU/mL	0.0 ng/mL	0.0
FSH	500 mIU/mL	0.0 ng/mL	0.0
HCG	1,000 mIU/mL	0.0 ng/mL	0.0

6.Hook Effect
No hook effect was observed up to 4,000 ng/mL in this assay.

Limitations

1. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot gurantee it will eliminate all the effects of that.

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8/2017

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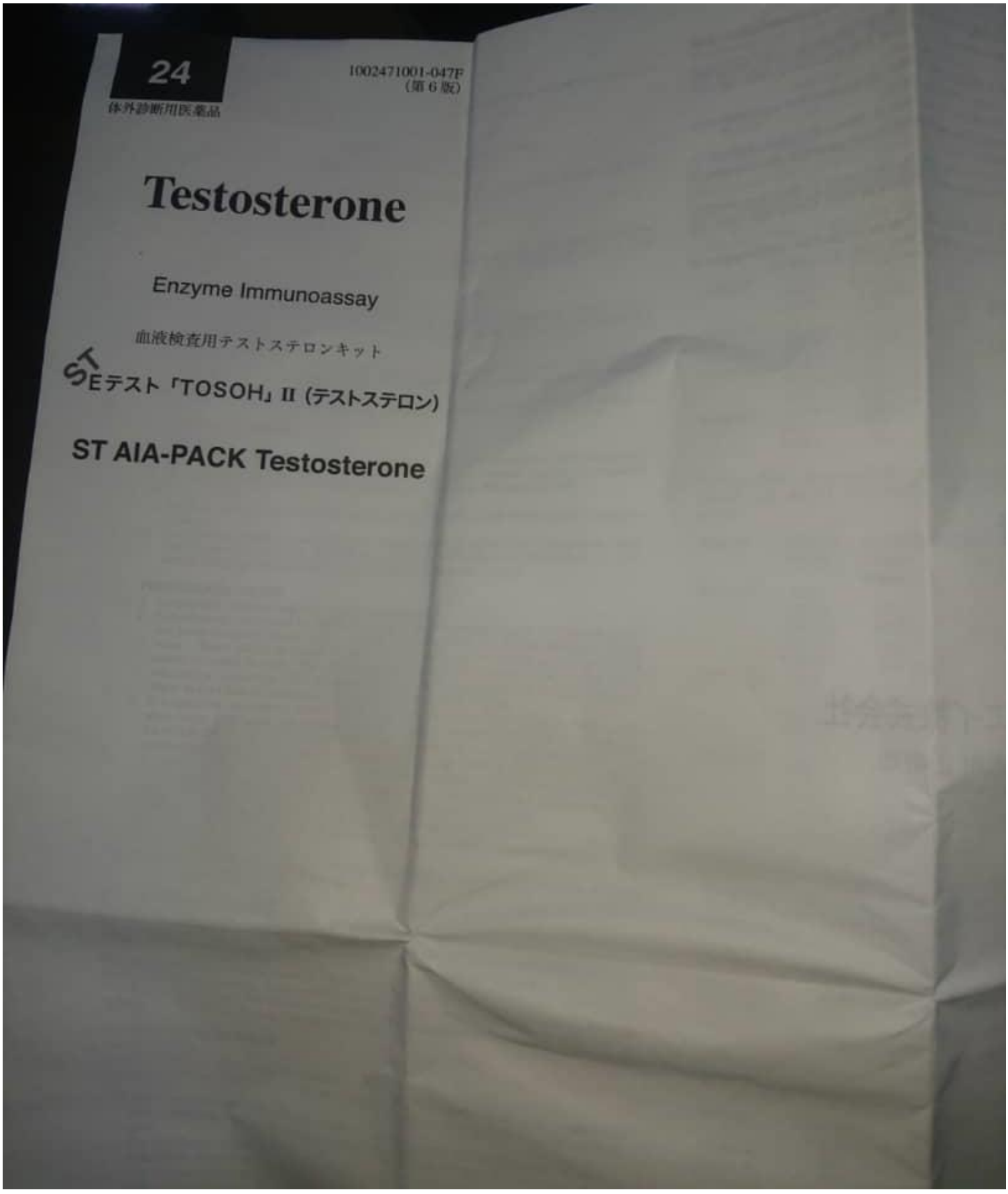
(For Export Only)

Appendix (2)


24
体外診断用医薬品
1002471001-047F
(第6版)
Testosterone
Enzyme Immunoassay
血液検査用テストステロンキット
STEテスト「TOSOH」II (テストステロン)
ST AIA-PACK Testosterone

1002471001-047F
Rev. 04/17

Attention

For U.S., Canada, and South America Customers: Please refer to the AIA Instructions for Use on CD for the appropriate information.

Para los Clientes en Estados Unidos, Canadá y Sur América: favor de referirse a los Instrucciónes de uso de AIA en Disco para la información apropiada.

Aos clientes da Estados Unidos, Canada e Aquérica do Sul: favor consultar a instrução de uso do AIA que estão em CD para informações adequadas.

Pour les clients en da États Unis, Canada et en Amérique du Sud: veuillez consulter Mode d' emploi AIA sur le CD pour l' information appropriée.

ST AIA-PACK Testosterone

For Quantitative Measurement of testosterone in Serum or Heparinized Plasma

NAME AND INTENDED USE

ST AIA-PACK Testosterone is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of testosterone in human serum or heparinized plasma on TOSOH AIA System Analyzers. Measurement of testosterone is used to aid in the diagnosis and management of conditions involving excess or deficiency of this analyte.

SUMMARY AND EXPLANATION OF TEST

Testosterone is one of the major male sex hormones produced by the interstitial cells of Leydig in the testes. The measurement of the total testosterone in serum can provide information to evaluate adrenal and testicular functions. It is useful for the diagnosis of hypergonadism *(1)* and hyperpituitarism *(2)* in men, and hirsutism *(3)*, menstrual disorders *(4)*, and polycystic ovarian syndrome *(5)* in women. It is also useful for the characterization and follow-up of some cancers such as testicular *(6)*, breast, ovarian, and adrenal tumors *(7-9)*.

PRINCIPLE OF THE ASSAY

ST AIA-PACK Testosterone is a competitive enzyme immunoassay which is performed entirely in the ST AIA-PACK Testosterone test cups. Testosterone present in the test sample competes with enzyme-labeled testosterone for a limited number of binding sites on the testosterone specific monoclonal antibody immobilized on a magnetic solid phase. The magnetic beads are washed to remove unbound enzyme-labeled testosterone and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled testosterone that binds to the beads is inversely proportional to the testosterone concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve.

MATERIAL PROVIDED (ST AIA-PACK Testosterone, Cat. No. 0025204)

5 trays x 20 test cups

Plastic test cups containing lyophilized twelve magnetic beads coated with mouse anti-testosterone monoclonal antibody and 45 µL of testosterone conjugated to bovine alkaline phosphatase with sodium azide as a preservative.

MATERIALS REQUIRED BUT NOT PROVIDED

The following materials are required to perform testosterone analysis using the ST AIA-PACK Testosterone (Cat. No. 0025204) on the TOSOH AIA System Analyzers. They are available separately from TOSOH.

Materials			Cat. No.
AIA Nex-IA or AIA-21			0018539
AIA Nex-IA or AIA-21 LA			0018540
AIA-1800 ST			0019836
AIA-1800 LA			0019837
AIA-2000 ST			0022100
AIA-2000 LA			0022101
AIA-600 II			0019014
AIA-600 II BCR			0019328
AIA-900			0022930
AIA-360			0019945
AIA-PACK SUBSTRATE SET II			0020968
AIA-PACK SUBSTRATE REAGENT II			
AIA-PACK SUBSTRATE RECONSTITUENT II			
ST AIA-PACK Testosterone CALIBRATOR SET			0025304
ST AIA-PACK Testosterone CALIBRATOR (1)	0	ng/dL	
ST AIA-PACK Testosterone CALIBRATOR (2)	40	ng/dL (approx.)	
ST AIA-PACK Testosterone CALIBRATOR (3)	100	ng/dL (approx.)	
ST AIA-PACK Testosterone CALIBRATOR (4)	350	ng/dL (approx.)	
ST AIA-PACK Testosterone CALIBRATOR (5)	900	ng/dL (approx.)	
ST AIA-PACK Testosterone CALIBRATOR (6)	2,200	ng/dL (approx.)	
ST AIA-PACK Testosterone SAMPLE DILUTING SOLUTION			0025504
AIA-PACK WASH CONCENTRATE			0020955
AIA-PACK DILUENT CONCENTRATE			0020956
SAMPLE CUPS			0018581
AIA-PACK DETECTOR STANDARDIZATION TEST CUP			0020970
AIA-PACK SAMPLE TREATMENT CUP			0020971
Additional Requirements for AIA Nex IA / AIA-21 *only*			
PIPETTE TIPS			0018552
PRELOADED PIPETTE TIPS			0018583
Additional Requirements for AIA-600 II, AIA-900, AIA-1800 and AIA-2000			
PIPETTE TIPS			0019215
TIP RACK			0019216
PRELOADED PIPETTE TIPS			0022103

Only materials obtained from TOSOH should be used. Materials obtained elsewhere should not be substituted since assay performance is characterized based entirely on TOSOH materials.

WARNINGS AND PRECAUTIONS

1. The ST AIA-PACK Testosterone is intended for *in vitro* diagnostic use only.
2. Inspect the packaging and the exterior of the aluminum pouch for any sign of damage before use. If any damages are visible, contact your local TOSOH sales representative.
3. Test cups from different lots or different assays shall not be mixed within a tray.
4. The ST AIA-PACK Testosterone contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
5. Human serum is not used in the preparation of this product; however, since human specimens will be used for samples and other quality control products in the lab may be derived from human serum, please use standard laboratory safety procedures in handling all specimens and controls.
6. Do not use beyond the expiration date.
7. For safe waste disposal, it is recommended that each laboratory complies with established laboratory procedures and local, state, and federal regulations.
8. After opening, the vial of ST AIA-PACK Testosterone SAMPLE DILUTING SOLUTION should be kept tightly sealed with a clean rubber cap. Sealing with any material may cause deterioration of the reagent.
9. The remaining sample diluting solution after use should not be mixed with another vial but be discarded to avoid contamination.
10. Serum, dust, sweat, or microorganism contamination may cause degradation of reconstituted substrate solution. Store in a clean environment, away from direct sunlight and ultraviolet light.
11. TOSOH recommends that a new pouch of the test cups should be used for calibration.

STORAGE AND STABILITY

All unopened materials are stable until the expiration date on the label when stored at the specified temperature.

Materials	Cat. No.
2-8 °C:	
ST AIA-PACK Testosterone	0025204
ST AIA-PACK Testosterone CALIBRATOR SET	0025304
ST AIA-PACK Testosterone SAMPLE DILUTING SOLUTION	0025504
AIA-PACK SUBSTRATE SET II	0020968
AIA-PACK WASH CONCENTRATE	0020955
AIA-PACK DILUENT CONCENTRATE	0020956
1-30 °C:	
AIA-PACK DETECTOR STANDARDIZATION TEST CUP	0020970
AIA-PACK SAMPLE TREATMENT CUP	0020971

After opening the aluminum pouch, ST AIA-PACK Testosterone test cups can be left on-board of the TOSOH AIA System Analyzers (18-25 °C) for a maximum of 10 days (10 x 24 hours). When stored over night at 2-8 °C, the test cups can be used for up to 30 days (30 cycles of 8 hours on-board and 16 hours in the refrigerator). Once the aluminum pouch is opened, the test cups must be used within 30 days.

ST AIA-PACK Testosterone CALIBRATOR SET must be kept tightly sealed and refrigerated at 2-8 °C. After opening, the calibrators should be used within 1 day.

After opening, ST AIA-PACK Testosterone SAMPLE DILUTING SOLUTION can be left on-board of the TOSOH AIA System Analyzers (18-25 °C) for a maximum of 3 days (3 x 24 hours). When stored over night at 2-8 °C, the sample diluting solution can be used for up to 9 days (9 cycles of 8 hours on board and 16 hours in the refrigerator). The sample diluting solution should not be used beyond 90 days after opening, even if it is sealed and stored in the refrigerator.

Reconstituted substrate solution is stable for 3 days at 18-25 °C or 30 days at 2-8 °C. Working diluent and wash solutions are stable for 30 days at 18-25 °C.

Reagents should not be used if they appear cloudy or discolored.

SPECIMEN COLLECTION AND HANDLING

1. Serum or heparinized plasma is required for the assay. EDTA and citrated plasma SHOULD NOT BE USED.
2. When using serum, a venous blood sample is collected aseptically without additives. Store at 18-25 °C until a clot has formed (usually 15-45 minutes), then centrifuge to obtain the serum specimen for assay.
3. When using heparinized plasma, a venous blood sample is collected aseptically with designated additive. Centrifuge and separate plasma from the packed cells as soon as possible.
4. Inadequate centrifugation or the presence of fibrin or particulate matter in the sample may cause an erroneous result.
5. Samples containing inhibitors of alkaline phosphatase may cause erroneous results.
6. Inspect all samples for air bubbles and foaming. Remove any air bubbles prior to assay.
7. Specimen types should not be used interchangeably during serial monitoring of an individual patient. Measured concentrations may vary slightly between sample types in certain patients.
8. Samples may be stored at 2-8 °C for up to 7 days prior to analysis. If the analysis cannot be done within 7 days, the sample should be stored frozen at –20 °C or below for up to 60 days.
9. Repeated freeze-thaw cycles should be avoided. Turbid serum samples or samples containing particulate matter should be centrifuged prior to testing. Prior to assay, slowly bring frozen samples to 18-25 °C and mix gently.
10. The sample required for analysis is 65 µL.

PROCEDURE

For the AIA Nex IA / AIA-21, AIA-600 II, AIA-900, AIA-1800, AIA-2000 and AIA-360, please refer to their Operator's Manual for detailed instructions.

I. Reagent Preparation

A) Substrate Solution

Bring all reagents to 18-25 °C before preparing the working reagent. Add the entire contents of the AIA-PACK SUBSTRATE RECONSTITUENT II (100 mL) to the lyophilized AIA-PACK SUBSTRATE REAGENT II and mix thoroughly to dissolve the solid material.

B) Wash Solution

Add the entire contents of the AIA-PACK WASH CONCENTRATE (100 mL) to approximately 2.0 L of CAP Class I water or the clinical laboratory reagent water (formerly NCCLS Type I) defined by CLSI GP40-A4-AMD guideline, mix well, and adjust the final volume to 2.5 L.

C) Diluent

Add the entire contents of the AIA-PACK DILUENT CONCENTRATE (100 mL) to approximately 4.0 L of CAP Class I water or the clinical laboratory reagent water (formerly NCCLS Type I) defined by CLSI GP40-A4-AMD guideline, mix well, and adjust the final volume to 5.0 L.

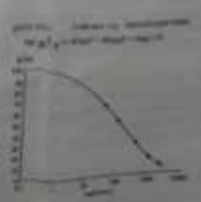
II. Calibration Procedure

A) Calibration Curve

The calibrators for use with the ST AIA-PACK Testosterone were compared to the USP reference material. The recovery of this reference material over the assay range is 84-133 % depending on concentration.

The calibration curve for ST AIA-PACK Testosterone is stable for up to 90 days. Calibration stability is monitored by quality control performance and is dependent on proper reagent handling and TOSOH AIA System maintenance according to the manufacturer's instructions. Recalibration may be necessary more frequently if controls are out of the established range for this assay or when certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, maintenance of the wash probe, or detector lamp adjustment or change). For further information regarding instrument operation, consult the TOSOH AIA System Operator's Manual.

A sample calibration curve from AIA-1800 follows and shows the algorithm used for calculating results.



B) Calibration Procedure

1. Refer to the appropriate TOSOH AIA System Operator's Manual for the procedural instructions.
2. Verify that both the calibrator lot and concentration numbers have been correctly entered into the software.
3. The ST AIA-PACK Testosterone CALIBRATOR SET is provided ready for use.
4. TOSOH recommends that all calibrators be run in triplicate.