



بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ



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**Shendi University**

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**Association Between Obesity and Serum Total Testosterone in  
Middle Aged Healthy Men in Shendi Locality**

A thesis submitted in partial fulfillment of the degree of Msc in medical  
laboratory sciences (clinical chemistry )

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قال تعالى:

﴿ الَّذِينَ يَحْمِلُونَ الْعَرْشَ وَمَنْ حَوْلَهُ يُسَبِّحُونَ بِحَمْدِ رَبِّهِمْ وَيُؤْمِنُونَ بِهِ  
وَيَسْتَغْفِرُونَ لِلَّذِينَ آمَنُوا رَبَّنَا وَسِعْتَ كُلَّ شَيْءٍ رَحْمَةً وَعِلْمًا فَاغْفِرْ لِلَّذِينَ تَابُوا  
وَاتَّبَعُوا سَبِيلَكَ وَقِهِمْ عَذَابَ الْجَحِيمِ ﴾

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الآية ( ٧ ) من سورة غافر



I would like to dedicate this work to.....

My parents whose affection, love, encouragement  
and prays of day and night make me able to get such  
success and honor.


*I dedicate this work to my wife .*

*To my teachers*

*To my lovely friends.*

*To anyone who encouraged*

*and supported me.*



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## Abstract

The body mass index is a measure of relative weight based on an individual's mass and height. Now a days the BMI is commonly used to classify underweight, overweight and obesity. It is calculated by dividing individual's weight in kilograms by his height in meters, then dividing the answer by his height again.  $BMI (kg/m^2) = \text{Body weight (kg)} / \text{Height (m)}^2$ .

This is a cross sectional, descriptive, case control study aimed to determine the association between serum total testosterone and BMI in middle aged healthy men. In the study, a total of 60 blood samples were collected. 20 middle aged healthy men with normal BMI , 20 middle aged healthy men overweight (BMI >25) and 20 middle aged healthy men obese (BMI >30) were selected randomly from volunteers was conducted Shendi locality in Nile River State in Sudan.

A volume of 5 ml blood were collected from each volunteer through vein puncture technique then displaced into Plain container .Each blood specimen was centrifuged at 3000 g for 5 minutes to obtain the serum for analysis to detection of testosterone levels by ELISA technique.

The study revealed Significant inverse correlation of serum total testosterone with BMI( $r = - 0.787$ , P value = 0.000) , WC( $r = - 0.717$ , P value = 0.000) and WHR ( $r = - 0.580$ , P value = 0.000) .

The study showed statistically significant effect on serum total testosterone levels with social status increased in single than married with (P value =0.000).

Also study showed insignificant relationship between age and serum total testosterone levels with (P value = 0.061).

The present finding statistically significant decreased of serum total testosterone levels with smoking with p. value ( 0.017 ) .

In this study there was no statistically significant effect on serum total testosterone levels with Exercise were mean P value (0.474).

## الخلاصة

مؤشر كتلة الجسم هو مقياس للوزن النسبي علي اساس وزن الفرد وطوله .واليوم يستخدم مؤشر كتلة الجسم عادة لتصنيف نقص الوزن و السمنة ويتم حسابه عن طريقه وزن الفرد بالكيلو جرام مقسوم علي مربع الطول بالامتار .مؤشر كتلة الجسم=وزن الجسم(كلغ) / الطول (م)<sup>2</sup> .

هذه الدراسة الوصفية اجريت لقياس العلاقة بين هرمون التستوستيرون و مؤشر كتلة الجسم (BMI) في ٦٠ عينة عشوائية من الرجال الاصحاء في متوسط العمر في محلية شندي ولاية نهر النيل .وتم قياس الطول والوزن لحساب مؤشر كتلة الجسم ثم تقسيمها الى ثلاث مجموعات متساويات (وزن طبيعي , وفوق الوزن الطبيعي وسمنة) . وايضا تم قياس طول الخصر والارداق لحساب ال WC, WHR.

وجدت هذه الدراسة تناسب عكسي بين هرمون التستوستيرون و زيادة الوزن وزيادة دهون البطن وايضا وجدت انا الاختلاف فالحالة الاجتماعية يؤثر علي مستوي هرمون التستوستيرون حيث يزيد عند غير المتزوجين عن المتزوجين وكانت الدالة الاحصائية (P.value 0.000) .

وايضا وجدت مستوي التعليم يؤثر علي هرمون التستوسترون كلما زاد مستوي التعليم يزيد مستوي الهرمون وكانت الدالة الاحصائية (p.value 0.018) .

خلصت الدراسة الي ان التدخين يؤثر علي مستوي التستوسترون وكانت الدالة الاحصائية (P.value 0.017)

و قد اظهرت الدراسة أن التمارين الرياضية ليس لها تاثير علي مستوي هرمون التستوسترون بدلالة احصائية(p.value 0.474) وايضا لم تجد تأثير للعمر علي مستوي الهرمون بدلالة احصائية (P.value 0.061)

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## List of abbreviation

Abbreviation	Meaning
ABP	Androgen-binding protein
AMH	Anti-Müllerian hormone
BMD	Bone mineral density
BMI	Body mass index
CVD	Cardio Vascular Disease
DM	Diabetes mellitus
ED	Erectile dysfunction
ELISA	Enzyme linked immune sorbent assay
FFA	Free Fatty Acids
FSH	Follicle stimulating hormone
FT	Free Testosterone
GnRH	Gonadotropin-releasing hormone
HCG	Human chorionic gonadotropin
HMG	Human menopausal gonadotropin
HPG	Hypothalamic-pituitary-gonadal
HPT	Hypothalamic pituitary testicular
LH	Luteinizing hormone
SD	Stander Deviation
SHBG	sex hormone binding globulin
SPSS	statistical package for social sciences
TT	total testosterone
WC	waist circumference
WHO	World Health Organization
WHR	Waist Hip Ratio

# Chapter One

**Introduction**

**Rationale**

**Objectives**

## 1.1 Introduction

**1.1.1 Male Sex Hormone** Sex steroid hormones are produced in the gonads: the testes in men and the ovaries in women. In humans, as opposed to rodents (e.g. mice and rats), sex steroid hormones are also produced from sex steroid precursors which originate from the adrenal cortex<sup>1</sup>. Sex steroid hormones include androgens, estrogens, and progesterone. In this thesis the focus lies on the effects of androgens and estrogens.<sup>(1)</sup>

**1.1.2 Testosterone:** Testosterone is the most important androgen in potency and quantity. Testosterone is synthesized and released by the Leydig cells that lie between the tubules and comprise less than 5% of the total testicular volume. Testosterone diffuses into the seminiferous tubules where it is essential for maintaining spermatogenesis. Some binds to an androgen-binding protein (ABP) that is produced by the Sertoli cells and is homologous to the sex-hormone binding globulin that transports testosterone in the general circulation. The ABP carries testosterone in the testicular fluid where it maintains the activity of the accessory sex glands and may also help to retain testosterone within the tubule and bind excess free hormone. Some testosterone is converted to estradiol by Sertoli cell-derived aromatase enzyme. Leydig cell steroidogenesis is controlled primarily by luteinizing hormone with negative feedback of testosterone on the hypothalamic-pituitary axis. The requirement of spermatogenesis for high local concentrations of testosterone means that loss of androgen production is likely to be accompanied by loss of spermatogenesis. Indeed, if testicular androgen production is inhibited by the administration of exogenous androgens then spermatogenesis ceases. This is the basis of using exogenous testosterone as a male contraceptive. Testosterone is converted to dihydrotestosterone by 5 $\alpha$ -reductase type 2. The androgen with the highest affinity for the androgen receptor. SRD5A2 deficiency illustrates the importance of dihydrotestosterone for external virilization, as individuals with this condition have normal male internal structures but their external genitalia are of

female appearance. There is now clear evidence that the human fetal testis and also the fetal adrenal gland is capable of testosterone biosynthesis during the first trimester. Regardless of the source of androgen production, the target tissue responds by male sexual differentiation of the external genitalia by the end of the first trimester. It is clear that testicular damage may result in loss of testosterone production or the loss of spermatogenesis or both. Loss of androgen production results in hypogonadism, the symptoms of which reflect the functions of testosterone. Male hypogonadism is defined as failure of the testes to produce normal amounts of testosterone, combined with signs and symptoms of androgen deficiency. Systemic testosterone levels fall by about 1% each year in men. Therefore, with increasing longevity and the aging of the population, the number of older men with testosterone deficiency will increase substantially over the next several decades. Serum testosterone levels decrease progressively in aging men, but the rate and magnitude of decrease vary considerably. Approximately 1% of healthy young men have total serum testosterone levels below normal; in contrast, approximately 20% of healthy men over age 60 years have serum testosterone levels below normal.<sup>(2)</sup>

**1.1.3 Relationship between serum testosterone and obesity:** as well as between testosterone and the metabolic syndrome. Low serum total testosterone predicts the development of central obesity and accumulation of intra-abdominal fat. Also, low total and free testosterone and SHBG levels are associated with an increased risk of developing the metabolic syndrome, independent of age and obesity. Lowering serum T levels in older men with prostate cancer treated with androgen deprivation therapy increases body fat mass. Conversely, high BMI, central adiposity, and the metabolic syndrome are associated with and predict low serum total and to a lesser extent free testosterone and SHBG levels. Because obesity suppresses SHBG and as a result total testosterone concentrations, alterations in SHBG confound the relationship between testosterone and obesity.<sup>(3)</sup>

## 1.2 Rationale

Approximately 30–40% of infertility cases can be attributed to problems with the male partner. Obesity and related concomitant metabolic abnormalities are among the proposed causes of male infertility. Metabolic syndrome has been characterized as a constellation of disorders, including Type 2 diabetes, coronary heart disease, and obesity with visceral abdominal fat distribution, dyslipidemia, hypertension and impaired glucose metabolism/insulin resistance. In the context of the ‘obesity epidemic’ in the Western world, three main biological mechanisms linking obesity to impaired male reproductive function. These mechanisms include hypogonadism, testicular heat-stress-/hypoxia-induced apoptosis and endocrine disruption by ‘obesogens’.<sup>(4)</sup>

So this study is going to associate between serum total testosterone and BMI in middle aged healthy men and correlate results with visceral abdominal fat distribution by estimate (WC), (WHR), and sociodemographic characteristics of participant. Results data will increase the knowledge about the association of testosterone levels and obesity in Sudan.

## **1.3 Objectives**

### **1.3.1 General objective:**

To assess the association between serum total testosterone and body Mass index BMI in middle aged healthy men

### **1.3.2 Specific objectives:**

- To correlate the testosterone levels with body Mass index (BMI), Waist Circumference (WC) and Waist hip ratio (WHR).
- To evaluate the association of testosterone levels with Waist Circumference (WC).
- To evaluate the association of testosterone levels with Waist hip ratio (WHR).
- To assess the association of testosterone levels with Age ,Smoking ,family history of obesity ,exercise and sociodemographic characteristics of participants.



# Chapter Two

**Literature review**

## 2. Literature Review

### 2.1 Male Sex Hormone

Sex steroid hormones are produced in the gonads: the testes in men and the ovaries in women. In humans, as opposed to rodents (e.g. mice and rats), sex steroid hormones are also produced from sex steroid precursors which originate from the adrenal cortex. Sex steroid hormones include androgens, estrogens, and progesterone. In this thesis the focus lies on the effects of androgens and estrogens.<sup>(1)</sup>

#### 2.1.1 Androgen synthesis in males

Adrenal androgens DHEA and androstenedione are produced in the zona reticulata and zona fasciculata of the adrenal cortex. Testosterone is produced by Leydig cells, which are found adjacent to the seminiferous tubules of the testes. In Leydig cells; LH initiates the production of pregnenolone. Pregnenolone is then converted to DHEA in a two-step process mediated by 17,20-lyase (17 $\alpha$ -hydroxylase). Because Leydig cells express high levels of 3 $\beta$ -HSD and 17 $\beta$ -HSD, DHEA is rapidly converted to testosterone via the intermediates androstenediol and androstenedione. Testosterone is converted to dihydrotestosterone (DHT) by the action of 5 $\alpha$ -reductase in target tissues; although it is about one-tenth as abundant as testosterone, it accounts for most of testosterone's biological action.<sup>(5)</sup> The major circulating androgen is testosterone, which is synthesized from cholesterol in the Leydig cells of the testis. The biosynthetic conversion of cholesterol to testosterone involves several discrete steps, of which the first one includes the transfer of cholesterol from the outer to the inner mitochondrial membrane by the steroidogenic acute regulatory (StAR) protein and the subsequent side chain cleavage of cholesterol by the enzyme P450<sub>scc</sub>. This conversion, resulting in the synthesis of pregnenolone, is the rate-limiting step in testosterone biosynthesis. Subsequent steps require several enzymes including 3 $\beta$ -hydroxysteroid

dehydrogenase, 17 $\alpha$ -hydroxylase/C17-20-lyase and 17 $\beta$ -hydroxysteroid dehydrogenase type 3.<sup>(6)</sup>

### 2.1.2 Testosterone

**Table (2-1) chemical structure of testosterone**

Chemical Names	<b>Testosterone; 58-22-0; Testosteron; Androderm; Mertestate; Sustanon</b>
Molecular Formula	<b>C<sub>19</sub>H<sub>28</sub>O<sub>2</sub></b>
Molecular Weight	288.431 g/mol

Testosterone is the most important androgen in potency and quantity. Testosterone is synthesized and released by the Leydig cells that lie between the tubules and comprise less than 5% of the total testicular volume. Testosterone diffuses into the seminiferous tubules where it is essential for maintaining spermatogenesis. Some binds to an androgen-binding protein (ABP) that is produced by the Sertoli cells and is homologous to the sex-hormone binding globulin that transports testosterone in the general circulation. The ABP carries testosterone in the testicular fluid where it maintains the activity of the accessory sex glands and may also help to retain testosterone within the tubule and bind excess free hormone. Some testosterone is converted to estradiol by Sertoli cell-derived aromatase enzyme. Leydig cell steroidogenesis is controlled primarily by luteinizing hormone with negative feedback of testosterone on the hypothalamic-pituitary axis. The requirement of spermatogenesis for high local concentrations of testosterone means that loss of androgen production is likely to be accompanied by loss of spermatogenesis. Indeed, if testicular androgen production is inhibited by the administration of exogenous androgens then spermatogenesis ceases. This is the basis of using exogenous testosterone as a male contraceptive. Testosterone is converted to dihydrotestosterone by 5 $\alpha$ -reductase type 2 (EC 1. 3. 1. 22, SRD5A2), the androgen with the highest affinity for the androgen receptor. SRD5A2 deficiency

illustrates the importance of dihydrotestosterone for external virilization, as individuals with this condition have normal male internal structures but their external genitalia are of female appearance. There is now clear evidence that the human fetal testis and also the fetal adrenal gland is capable of testosterone biosynthesis during the first trimester. Regardless of the source of androgen production, the target tissue responds by male sexual differentiation of the external genitalia by the end of the first trimester. It is clear that testicular damage may result in loss of testosterone production or the loss of spermatogenesis or both. Loss of androgen production results in hypogonadism, the symptoms of which reflect the functions of testosterone. Male hypogonadism is defined as failure of the testes to produce normal amounts of testosterone, combined with signs and symptoms of androgen deficiency. Systemic testosterone levels fall by about 1% each year in men. Therefore, with increasing longevity and the aging of the population, the number of older men with testosterone deficiency will increase substantially over the next several decades. Serum testosterone levels decrease progressively in aging men, but the rate and magnitude of decrease vary considerably. Approximately 1% of healthy young men have total serum testosterone levels below normal; in contrast, approximately 20% of healthy men over age 60 years have serum testosterone levels below normal.<sup>(2)</sup>

#### **2.1.2.1. Testosterone Production and Release:**

##### **2.1.2.1.1 Testosterone Production:**

Testosterone (17 $\beta$ -hydroxy-4-androstene-3-one) is a 0.288kD C<sub>19</sub> steroid hormone produced from cholesterol via a series of conversions catalyzed by specific enzymes; this process takes approximately 20–30 minutes from initiation to final product. Several of the intermediates in this process are hormones with their own physiological actions and include progesterone, dihydroepiandrosterone (DHEA) and androstenedione; the former is involved in the female reproductive cycle and the latter two have weak androgenic-anabolic effects, the primary production site

of testosterone is the Leydig cells. These cells are only found in the testes, which largely explain the approximately 10-fold higher circulating testosterone concentrations in men compared with women. Testosterone is also produced in smaller quantities in the ovaries and the zona reticularis of the adrenal cortex, This testosterone formation is mainly spillover from the production of other hormones such as cortisol and aldosterone (in the adrenal glands) that share some precursors with testosterone, and estradiol (in the ovaries) for which testosterone itself is a precursor. These shared precursors help explain how the adrenal gland and the ovaries can produce testosterone despite the absence of Leydig cells in these tissues. This spillover, along with peripheral conversion of androgens, is the primary source of testosterone in females and adolescent boys. The absence of functioning cells dedicated to testosterone production and release prevents large acute increases in circulating testosterone in females and adolescent boys in response to exercise. Although peripheral production (e.g. in muscle tissue) of testosterone occurs .this production does not appear to be affected by resistance exercise in humans. <sup>(7)</sup>

#### **2.1.2.2 Testosterone Metabolism:**

Metabolism of androgens have been undertaken in this laboratory in order to get additional data useful for determinations of their secretion and production rates in viva , and to find a biochemical explanation of their different biological activities. Testosterone' and androstenedione are both secreted in man. testosterone is known as the most potent natural androgen and androstenedione as a very weak one, paradoxically their metabolism is believed to be identical from both the qualitative and the quantitative points of view. When either of these hormones is given as a radioactive tracer, nearly 100% of the injected radioactivity appears in the urine, and 20 to 70% is found as radioactive androsterone and 5@-androsterone. The reversible reaction, androstenedione = testosterone, has been demonstrated in the liver, the kidney, and other tissues. It is considered that testosterone is oxidized to

androstenedione and then reduced to androsterone and 5 $\beta$ -androsterone, which are conjugated with both glucuronic and sulfuric acid before excretion in the urine. These reactions may be called the “17-ketonic pathway” of testosterone metabolisms.<sup>(8)</sup>

### **2.1.2.3 Testosterone Transport:**

Humans have a plasma  $\beta$ -globulin that binds testosterone with specificity, relatively high affinity, and limited capacity sex-hormone-binding globulin (SHBG), which is produced in the liver. Its production is increased by estrogens (women have twice the serum concentration of SHBG as men), certain types of liver disease, and hyperthyroidism; it is decreased by androgens, advancing age, and hypothyroidism. Since SHBG and albumin bind 97% to 99% of circulating testosterone, only a small fraction of the hormone in circulation is in the free (biologically active) form. The primary function of SHBG may be to restrict the free concentration of testosterone in the serum. Testosterone binds to SHBG with higher affinity than does estradiol. Therefore, a change in the level of SHBG causes a greater change in the free testosterone level than in the free estradiol level. Because the metabolic clearance rates of these steroids are inversely related to the affinity of their binding to SHBG, estrogen is cleared more rapidly than estradiol, which in turn is cleared more rapidly than testosterone or DHT.<sup>(9)</sup>

### **2.1.2.4 Effects of testosterone :**

The complex effects of testosterone, investigators found depend partly on its conversion in the body to a type of estrogen. The insights will help guide the development of better ways to diagnose and treat men who don't produce enough natural testosterone. Testosterone is a sex hormone that plays important roles in the body. In men, it's thought to regulate sex drive (libido), bone mass, fat distribution, muscle mass and strength, and the production of red blood cells and sperm. A small amount of circulating testosterone is converted to estradiol, a form of estrogen. As men age, they often make less testosterone, and so they produce less

estradiol as well. Thus, changes often attributed to testosterone deficiency might be partly or entirely due to the accompanying decline in estradiol. Testosterone was first used as a clinical drug as early as 1937, but with little understanding of its mechanisms. The hormone is now widely prescribed to men whose bodies naturally produce low levels. But the levels at which testosterone deficiency become medically relevant still aren't well understood. Normal testosterone production varies widely in men, so it's difficult to know what levels have medical significance. The hormone's mechanisms of action are also unclear.<sup>(10)</sup>

#### **2.1.2.5 Testosterone and spermatogenesis:**

Testosterone is essential to maintain spermatogenesis and male fertility. In the absence of testosterone stimulation, spermatogenesis does not proceed beyond the meiosis stage. After withdrawal of testosterone, germ cells that have progressed beyond meiosis detach from supporting Sertoli cells and die, whereas mature sperm cannot be released from Sertoli cells resulting in infertility. The classical mechanism of testosterone action in which testosterone activates gene transcription by causing the androgen receptor to translocate to and bind specific DNA regulatory elements does not appear to fully explain testosterone regulation of spermatogenesis. This review discusses two non-classical testosterone signaling pathways in Sertoli cells and their potential effects on spermatogenesis. Specifically, testosterone-mediated activation of phospholipase C and calcium influx into Sertoli cells is described. Also, testosterone activation of Src, EGF receptor and ERK kinases as well as the activation of the CREB transcription factor and CREB-mediated transcription is reviewed. Regulation of germ cell adhesion to Sertoli cells and release of mature sperm from Sertoli cells by kinases regulated by the non-classical testosterone pathway is discussed. The evidence accumulated suggests that classical and non-classical testosterone signaling contribute to the maintenance of spermatogenesis and male fertility.<sup>(11)</sup>

### **2.1.2.6 Effect of age on testosterone :**

Testosterone and gonadotropins. Several studies demonstrate that the serum testosterone concentration declines with increasing age. In one cross-sectional study of 83 healthy men, the free testosterone concentration declined by age 80 to approximately 40% of that at age 20, but because the serum concentration of sex hormone binding globulin (SHBG) increased during this age span, the serum concentration of total testosterone declined only to approximately 80% of that at age 20 . In another cross-sectional study of 71 healthy men, aged 26–90 yr, living in similar circumstances, the 33 younger men had a mean free testosterone concentration of  $10.7 \pm 3.4$  ng/dL (SD) and the 38 older men had a mean free testosterone concentration of 5.8 ng/dL ( $P < 0.01$ ) . Total serum testosterone concentrations in the two groups were  $659 \pm 201$  ng/dL and  $490 \pm 160$  ng/dL, respectively ( $P < 0.01$ ). In a third cross-sectional study in 810 healthy men mostly between 50 and 84 yr old, the serum bioavailable testosterone concentration declined by approximately 55–60%, but the serum total testosterone concentration did not decline at all .<sup>(12)</sup>

### **2.1.3 Hypogonadism:**

Male hypogonadism is characterized by androgen deficiency and infertility. Hypogonadism can be caused by disorders at the hypothalamic or pituitary level (hypogonadotropic forms) or by testicular dysfunction (hypergonadotropic forms). Testosterone substitution is necessary in all hypogonadal patients, because androgen deficiency causes slight anemia, changes in coagulation parameters, decreased bone density, muscle atrophy, regression of sexual function and alterations in mood and cognitive abilities. Androgen replacement comprises injectable forms of testosterone as well as implants, transdermal systems, sublingual, buccal and oral preparations. Transdermal systems provide the pharmacokinetic modality closest to natural diurnal variations in testosterone levels. New injectable forms of testosterone are currently under clinical evaluation



(testosterone undecanoate, testosterone buciclate), allowing extended injection intervals. If patients with hypogonadotropic hypogonadism wish to father a child, spermatogenesis can be initiated and maintained by gonadotropin therapy (conventionally in the form of human chorionic gonadotropin (hCG) and human menopausal gonadotropin (hMG) or, more recently, purified or recombinant follicle stimulating hormone (FSH). Apart from this option, patients with disorders at the hypothalamic level can be stimulated with pulsatile gonadotropin-releasing hormone (GnRH). Both treatment modalities have to be administered on average for 7–10 months until pregnancy is achieved. In individual cases, treatment may be necessary for up to 46 months. Testosterone treatment is interrupted for the time of GnRH of gonadotropin therapy, but resumed after cessation of this therapy.<sup>(13)</sup>

The clinical picture of male hypogonadism depends on whether testicular deficiency develops before or after puberty. In adults, if it is due to testicular disease, circulating gonadotropin levels are elevated (hypergonadotropic hypogonadism); if it is secondary to disorders of the pituitary or the hypothalamus (eg. Kallmann syndrome), circulating gonadotropin levels are depressed (hypogonadotropic hypogonadism). If the endocrine function of the testes is lost in adulthood, the secondary sex characteristics regress slowly because it takes very little androgen to maintain them once they are established. The growth of the larynx during adolescence is permanent, and the voice remains deep. Men castrated in adulthood suffer some loss of libido, although the ability to copulate persists for some time. They occasionally have hot flushes and are generally more irritable, passive, and depressed than men with intact testes. When the Leydig cell deficiency dates from childhood, the clinical picture is that of eunuchoidism. Eunuchoid individuals over the age of 20 are characteristically tall, although not as tall as hyper pituitary giants, because their epiphyses remain open and some growth continues past the normal age of puberty. They have narrow shoulders and small muscles, a body configuration resembling that of the adult female. The

genitalia are small and the voice high-pitched. Pubic hair and auxiliary hair are present because of adrenocortical androgen secretion. However, the hair is sparse, and the pubic hair has the female “triangle with the base up” distribution rather than the “triangle with the base down” pattern (male escutcheon) seen in normal males.<sup>(14)</sup>

### **2.1.3.1 Causes of male hypogonadism:**

#### **Primary hypogonadism**

- Congenital anorchidism
- Cryptorchidism
- Mumps orchitis
- Genetic and developmental conditions: Klinefelter syndrome,
- androgen receptor and enzyme
- Defects, Sertoli cell only syndrome
- Radiation treatment/chemotherapy
- Testicular trauma
- Autoimmune syndromes (anti-Leydig cell disorders)

#### **Secondary hypogonadism**

- Genetic conditions: Kallmann’s syndrome, Prader-Willi
- syndrome
- Pituitary tumours, granulomas, abscesses
- Hyperprolactinemia
- Cranial trauma
- Radiation treatment
- Various medications
- Mixed (primary and secondary) hypogonadism\*
- Alcohol abuse
- Ageing

- Chronic infections (HIV)
- Corticosteroid treatment
- Hemochromatosis
- Systemic disease (liver failure, uremia, sickle-cell disease)
- \*Mixed hypogonadism is often included within the secondary.<sup>(15)</sup>

### **2.1.3.2 Etiology:**

Testosterone (T) levels (<300 ng/dl) together with X1 clinical symptom or sign. Symptoms of post pubertal hypogonadism include sexual dysfunction, such as reduced libido, erectile dysfunction (ED), diminished penile sensation, difficulty attaining orgasm, as well as reduced ejaculate with orgasm; reduced energy, vitality, or stamina; depressed mood or diminished sense of well-being; increased irritability; difficulty concentrating and other cognitive problems; and/or hot flashes in some cases of acute onset. Signs of hypogonadism include anemia; muscle wasting (sarcopenia); reduced bone mass or bone mineral density (BMD); absence or regression of secondary sex characteristics; Hypogonadism is characterized by low serum abdominal adiposity (i.e. ‘pot belly’ obesity); and/or oligospermia or azoospermia. A number of hypothalamic-pituitary-gonadal (HPG) axis defects may induce hypogonadism The term primary (hypergonadotropic) hypogonadism refers to testicular disorders and is characterized by low serum T despite high levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Causes of primary hypogonadism include genetic conditions (e.g. Klinefelter syndrome, gonadal dysgenesis); anatomic defects; infection; tumor; injury; iatrogenic causes (surgery or certain medications); and/ or alcohol abuse.<sup>(16)</sup>

### **2.1.3.3 Laboratory diagnosis of male hypogonadism in the setting of systemic diseases:**

The Endocrine Society, in their recently published Clinical Practice Guidelines, recommends that when a patient presents with signs and symptoms suggestive of androgen deficiency, measurement of morning serum total testosterone levels

should be performed. A patient who has a total testosterone level below 300 ng/dL is likely to be hypogonadal. In patients who have levels between 200 and 400 ng/dL, the test should be repeated along with measurement of free testosterone. Measurement of free testosterone or sex-hormone-binding globulin (SHBG) levels is helpful in determining bioavailable testosterone because many systemic conditions are associated with changes in SHBG levels. Specifically, increases in SHBG can be detected in hepatic cirrhosis, hyperthyroidism, HIV infection, and anticonvulsant use; reductions in SHBG are noted with moderate obesity, low protein states (nephrotic syndrome), hypothyroidism, hyperinsulinism, and glucocorticoid use.<sup>(17)</sup>

## **2.2 Obesity**

Obesity is defined as having a body mass index (BMI) of 30 or more. BMI is a calculation that takes a person's weight and height into account. However, BMI does have some limitations.<sup>(18)</sup>

Obesity, as defined by World Health Organization (WHO), is excess weight gain for a given height. Obesity now represents one of the most common medical conditions in the United States. Obesity among adults in the developed world is defined as a body mass index (BMI) over 30 kgm<sup>2</sup>, and morbid obesity constitutes a BMI of 40 or higher. Almost a third of adults in the United States are now obese on the basis of current measured weights and heights.<sup>(19)</sup>

Obesity is an increasing serious public health problem. The incidence of overweight and obesity is reaching pandemic levels. In addition, overweight and obesity are associated with a high burden of specific and chronic comorbidities such as diabetes and renal dysfunction. During recent years, different studies have highlighted the resolution of diabetes in obese patients after treatment with bariatric surgery as well as an amelioration of renal function and other associated comorbidities. Hypogonadism and sub fertility are also other prevalent endocrine dysfunctions among obese people, and its improvement after weight loss maybe

another additional benefit of treatment and can be placed in this category of modifiable comorbidities associated to obesity. Obesity may directly alter gonadal function along with other comorbidities present in the obese subject, such as diabetes, which may contribute to affect sexual function. There are a few published reports evaluating the effect of sustained weight loss on sexual hormones in male morbidly obese patients [defined by body mass index (BMI) higher than 40 kg/m<sup>2</sup>]. Most of them have found that obese males show decreased sexual quality of life, reduced fertility, and hormonal changes including decreased testosterone levels (both free and total), increased estradiol in comparison to the general population as well as a decrease of the sex hormone binding globulin (SHBG). The changes of gonadotropin serum levels observed in obesity are more controversial but inappropriate normal levels, suggesting a hypogonadotropic hypogonadism situation is generally found.<sup>(20)</sup>

### **2.2.1 Obesity and the adipose tissue**

Obesity, characterized by an increase in the adipose tissue mass, is a key pathological contributor to the metabolic syndrome. The primary function of the adipose tissue is for energy storage. In addition, adipose tissues also play an important role in systemic glucose homeostasis. In adult mammals, the major bulk of adipose tissue is a loose association of lipid-filled adipocytes, which are held in a framework of collagen (stroma)-containing vascular cells, fibroblastic connective tissue cells, leukocytes, macrophages and pre-adipocytes.<sup>14</sup> The composition of white adipose tissue includes 60–85% lipid, 90–99% of which are triglycerides. Adipose tissue also contains small amounts of free fatty acids (FFA), triglycerides, cholesterol and phospholipids.<sup>(21)</sup>

### **2.2.2. Relationship between serum testosterone and obesity**

Relationship between serum testosterone and obesity as well as between testosterone and the metabolic syndrome. Low serum total testosterone predicts the development of central obesity and accumulation of intra-abdominal fat. Also, low

total and free testosterone and SHBG levels are associated with an increased risk of developing the metabolic syndrome, independent of age and obesity . Lowering serum T levels in older men with prostate cancer treated with androgen deprivation therapy increases body fat mass . Conversely, high BMI, central adiposity, and the metabolic syndrome are associated with and predict low serum total and to a lesser extent free testosterone and SHBG levels .Because obesity suppresses SHBG and as a result total testosterone concentrations, alterations in SHBG confound the relationship between testosterone and obesity.<sup>(3)</sup>

Obesity has been associated with various endocrine abnormalities, both in men and women. It is well known that plasma testosterone levels in the obese decline with increasing body weight, particularly in men with central obesity Testosterone levels are lower in obese men because of the decreased levels of sex-hormone binding globulin (SHBG); these hormone changes get worse in massively obese men, creating a relative hypogonadal state, which seems to be closely related to insulin resistance.<sup>(22)</sup>

It has been suggested that obesity-associated male hypogonadism is related to the central inhibition of gonadotropin secretion, but the mechanisms are yet unknown. One hypothesis is that hypothalamic GnRH secretion may be inhibited by the increased estradiol from excessive peripheral conversion of testosterone in the adipose tissue. However, not all studies have shown a decrease in estradiol levels after weight loss. On the other hand, inhibin B and anti-Müllerian hormone (AMH), which reflect Sertoli cell function, have been less explored in male obesity.<sup>(20)</sup>

### **2.2.2.1 Obesity, low testosterone levels**

In male subjects total testosterone blood concentrations are inversely correlated with body weight. Therefore, obese male subjects have lower mean total testosterone (TT) concentrations than normal weight healthy controls. Several factors have been proposed to account for the decreased plasma TT levels in obese

patients, including decreased sex hormone binding globulin (SHBG) synthesis<sup>8</sup> and decreased pituitary gonadotropin secretion and pulse amplitude.<sup>(23)</sup>

#### **2.2.2.2 Low Testosterone And Obesity Beyond Testosterone Fat Interaction**

Because of its association with sarcopenia, low testosterone may compound the effect of increasing fat mass by making it more difficult for obese men to lose weight via exercise. Conversely, obesity in itself contributes to loss of muscle mass and function, thus escalating the effects of sarcopenia on mobility disability and functional impairment, a concept known as ‘sarcopenic obesity’. Indeed, pro-inflammatory cytokines released by adipose tissue may contribute to loss of muscle mass and function, leading to inactivity and further weight gain in a vicious cycle. Sarcopenic obesity, a phenotype recapitulated in men receiving ADT for prostate cancer, may not only be associated with functional limitations, but also aggravate the metabolic risks of obesity; the association of low testosterone with sarcopenia may be an additional mechanism linking low testosterone to insulin resistance beyond its relationship to increased visceral fat.<sup>(24)</sup>

#### **2.2.3 Body Mass Index**

The BMI is a measure of relative weight based on an individual's mass and height. Nowadays the BMI is commonly used to classify underweight, overweight and obesity. Moreover, it is adopted by the British government in an effort to promote healthy eating .It is calculated by dividing individual’s weight in kilograms by his height in meters, then dividing the answer by his height again.

$$\text{BMI (kg/m}^2\text{)} = \text{Body weight (kg)} / \text{Height (m)}^2. \text{ }^{(25)}$$

In recent years, there has been increasing speculation over which measure of overweight and obesity is best able to discriminate those individuals who are at increased cardiovascular risk. Body mass index (BMI) is used by the World Health Organization to define severity of overweight and obesity across populations. But increasingly, measures of central adiposity, namely waist circumference (WC) and waist-to-hip ratio (WHR), have been adopted as more accurate predictors of

obesity-related cardiovascular risk and have replaced BMI in several definitions for clinical diagnosis of metabolic syndrome.<sup>(26)</sup>

## **2.3 Infertility**

### **2.3.1 Definition:**

Infertility is defined as the incapacity to fulfill pregnancy after a reasonable time of sexual intercourse with no contraceptive measures taken. The terms sterility and infertility are sometimes used interchangeably and at times define different populations. In the Spanish literature, the definition of the word sterility is the difficulty to fulfill pregnancy, whilst the term infertility is used when pregnancy develops but is interrupted at some point; hence, the term is used as a synonym of recurrent miscarriages. On the contrary, in the English literature the term infertile refers to a couple that fails in achieving pregnancy, either because of the impossibility to become pregnant through natural means (sterility) or whenever the possibilities exist but pregnancy does not occur (sub fertility) or if pregnancy does develop but does not lead to a live newborn. In contrast, the fertile population is defined as those who do become pregnant after some reasonable time of regular sexual intercourse.<sup>(27)</sup>

Infertility (clinical definition) is currently defined as 1 year of unwanted non-conception with unprotected intercourse in the fertile phase of the menstrual cycles.<sup>(28)</sup>

### **2.3.2 Mechanisms of obesity-induced male infertility**

Approximately 30–40% of infertility cases can be attributed to problems with the male partner. Obesity and related concomitant metabolic abnormalities are among the proposed causes of male infertility. Metabolic syndrome has been characterized as a constellation of disorders, including Type 2 diabetes, coronary heart disease, and obesity with visceral abdominal fat distribution, dyslipidemia, hypertension and impaired glucose metabolism/insulin resistance. In the context of the ‘obesity epidemic’ in the Western world, this paper discusses three main biological



mechanisms linking obesity to impaired male reproductive function. These mechanisms include hypogonadism, testicular heat stress-/hypoxia-induced apoptosis and endocrine disruption by ‘obesogens.’<sup>(4)</sup>

## **2.4 Previous Studies**

### **2.4.1 Association between serum total testosterone and Body Mass Index in middle aged healthy men**

Mean ( $\pm$  SD) age of the subjects included in this study was 38.7 ( $\pm$  6.563) years mean ( $\pm$  SD) total testosterone was 15.92 ( $\pm$ 6.322)nmol/L. The mean ( $\pm$  SD) BMI, and WHR were 24.95 ( $\pm$ 3.828) kg/m<sup>2</sup> and 0.946 ( $\pm$ 0.0474) respectively. Statistically significant differences were observed in the mean values of BMI and WHR for the two groups of testosterone. Significant inverse correlation of serum total testosterone with BMI( $r = -0.311$ ,  $p = 0.000$ ) was recorded in this study. However testosterone was not significantly correlated with waist/hip ratio.( $r = -0.126$ ,  $p = 0.076$ ) Middle age men working at DUHS who have low level of serum total testosterone are more obese than individuals with normal total testosterone level.<sup>(29)</sup>

### **2.4.2 Relationship between sex steroid hormones, sex hormone-binding globulin, leptin, insulin and insulin resistance in obese men.**

There are interactions between low SHBG and insulin concentrations, and a relationship between low total and free testosterone concentrations with increased insulin resistance in obese men. We found that T and SHBG serum concentrations decreased as the BMI increased. Similar results have been reported by others. There is a possible role of sexual steroid hormones and SHBG in metabolic disturbances associated with obesity. Obesity is associated with a decrease in T, FT, SHBG levels. There is also an inverse correlation between the FT levels and central obesity.<sup>(22)</sup>

### **2.4.3 Body weight loss reverses obesity-associated hypogonadotropic hypogonadism: a systematic review and meta-analysis**

Out of 266 retrieved articles, 24 were included in the study. Of the latter, 22 evaluated the effect of diet or bariatric surgery, whereas two compared diet and bariatric surgery. Overall, both a low calorie diet and bariatric surgery are associated with a significant (P: 0.0001) increase in plasma sex hormone-binding globulin-bound and -unbound testosterone levels (total testosterone (TT), with bariatric surgery being more effective in comparison with the low-calorie diet (TT increase: 8.73 (6.51–10.95) VS 2.87 (1.68–4.07) for bariatric surgery and the low calorie diet, respectively; both P: 0.0001 VS baseline). Androgen rise is greater in those patients who lose more weight as well as in younger, non-diabetic subjects with a greater degree of obesity. Body weight loss is also associated with a decrease in estradiol and an increase in gonadotropins levels. Multiple regression analysis shows that the degree of body weight loss is the best determinant of TT rise (BZ2.50G0.98, PZ0.029).<sup>(30)</sup>

### **2.4.4 Obesity, low testosterone levels and erectile dysfunction**

Available data clearly show a relationship between obesity, low testosterone levels and ED. Obesity adversely affects endothelial function and lowers serum testosterone levels through the development of insulin resistance and metabolic syndrome. Metabolic disturbances as well as production of cytokines and adipokines by inflamed fat cells may be causal factors in the development of ED. The onset of ED and the associated risk of CVD may be delayed through lifestyle modifications that affect obesity, such as diet and exercise. Very low testosterone levels contribute to the development of ED in obesity, metabolic syndrome and type 2 diabetes mellitus. Whether or not testosterone treatment of obese individuals decreases the risk of metabolic syndrome and type 2 diabetes mellitus remains controversial.<sup>(21)</sup>

#### **2.4.5 Lowered testosterone in male obesity: mechanisms, morbidity and management:**

With increasing modernization and urbanization of Asia, much of the future focus of the obesity epidemic will be in the Asian region. Low testosterone levels are frequently encountered in obese men who do not otherwise have a recognizable hypothalamic pituitary testicular (HPT) axis pathology. Moderate obesity predominantly decreases total testosterone due to insulin Resistance associated reductions in sex hormone binding globulin. More severe obesity is additionally associated with reductions in free testosterone levels due to suppression of the HPT axis. Low testosterone by itself leads to increasing adiposity, creating a self-perpetuating cycle of metabolic complications. Obesity associated hypotestosteronemia is a functional, non permanent state, which can be reversible, but this requires substantial weight loss. While testosterone treatment can lead to moderate reductions in fat mass, obesity by itself, in the absence of symptomatic androgen deficiency, is not an established indication for testosterone therapy. Testosterone therapy may lead to a worsening of untreated sleep apnea and compromise fertility. Whether testosterone therapy augments diet and exercise induced weight loss requires evaluation in adequately designed randomized controlled clinical trials.<sup>(24)</sup>

#### **2.4.6 Influence of Some Biological Indexes on Sex Hormone- Binding Globulin and Androgen Levels in Aging or Obese Males**

Respectively, has been reported in studies involving groups of men covering a wide range of BMI (3, 21-25). Our data show that this inverse correlation exists in non obese (BMI, 26) healthy men ( $P < 0.001$ ) as well as in our obese population. The consistency of this correlation found in all studies as well as the fact that insulin is known to decrease SHBG synthesis suggest a causal relationship between obesity and the decrease in SHBG levels, probably via the accompanying hyperinsulinism. As BMI often increases.<sup>(31)</sup>

# Chapter Three

## **Materials and Methods**

### **3. Materials and Methods**

#### **3.1 Study design:**

This is a cross sectional, descriptive, case control study conducted in River Nile State (Shendi Locality) from April 2018 to August 2018, to assess the association between serum total testosterone and BMI in middle aged healthy men.

#### **3.2 Study area:**

This study was conducted Shendi in River Nile State in Sudan

#### **3.3 Study populations:**

Middle aged healthy men (18-50) years

#### **3.4 Sample size:**

The study included 60 individuals, 20 Middle Aged Healthy Men with Normal BMI, 20 Middle aged healthy men overweight (BMI >25) and 20 middle aged healthy Men obese (BMI >30) were selected randomly.

#### **3.5 Study Criteria:**

##### **3.5.1 Inclusion criteria:**

Middle aged healthy men with different weight (normal, overweight and obese).

##### **3.5.2 Exclusion criteria:**

- Adult males who had diseases which may affect testosterone levels
- Adult males who refused to participate.

#### **3.6 Data collection:**

Data were collected by using a questionnaire and filled by the investigator during each time when blood samples were collected.

#### **3.7 Methodology:**

##### **3.7.1 Collection of blood specimens:**

A volume of 5 ml blood were collected from each patient through venipuncture technique then displaced into Plain container.

### 3.7.2 Sample processing:

Each blood specimen was centrifuged at 3000 g for 5 minutes to obtain the serum. The later was gently collected into plain container and stored at -20 °C until the serological analysis.

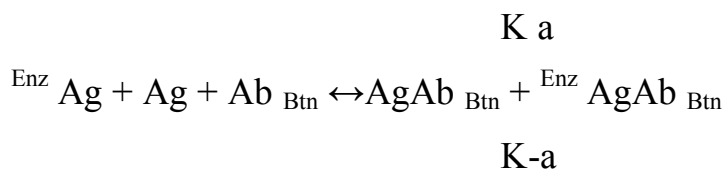
### 3.7.3 Enzyme linked immune sorbent assay (ELISA) processing

The specimens were analyzed for quantitative detection of testosterone levels by commercially available enzyme-linked immune sorbent assay testosterone Micro plate Enzyme Immunoassay, **BXE0862A** (96 tests)” kit (Fortress Diagnostics Limited, unit 2C Antrim Technology Park, Antrim, BT41 1QS United Kingdom). The assays were performed following the instructions of the manufacturer. According to the information included in the kit’s insert, the immunoassay used has 98.0% sensitivity and 99.3% specificity.

#### 3.7.3.1 Principle of Enzyme linked immune sorbent assay (ELISA) for detection of testosterone levels: Competitive Enzyme Immunoassay (Type 7)

The essential reagents required for a enzyme immunoassay include antibody, enzyme-antigen

conjugate and native antigen. Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme - a/ka = Equilibrium Constant.



Ab<sub>Btn</sub> = Biotinylated Antibody ( Constant Quantity)

Ag = Native Antigen ( Vairable Quantity)

EnzAg= Enzyme- antigen Conjugate (Constant Quantity)

AgAb<sub>Btn</sub>= Antigen – Antibody Complex

Enz AgAb Btn= Enzyme-antigen Conjugate-Antibody Complex

K a = Rate Constant of Association

$K_{-a}$  = Rate Constant of Disassociation

$K = k_a / K_{-a}$  = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.

$AgAb_{Btm} + {}^{Enz}AgAb_{Btm} + Streptavidin\ CW \rightleftharpoons immobilized\ complex$

Streptavidin CW = Streptavidin immobilized on well

Immobilized complex = sandwich complex bound to the solid surface.

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

### **3.7.3.2 Reagent Preparation:**

1. Working Enzyme Reagent – was made fresh for each run. 1 volume of the Testosterone Enzyme reagent was mixed with 10 Volumes of the Enzyme Buffer, and was used immediately.
2. Wash Buffer: contents of wash solution was Diluted to 1000ml with distilled water in a suitable storage container.

### **3.7.3.3 Procedure of Enzyme linked immunosorbent assay (ELISA) for detection of testosterone levels:**

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27°C).

1. The microplate's wells were formatted for each serum reference, control and patient specimen to be assayed.
2. 0.050ml (50 $\mu$ l) of the appropriate serum reference, control and specimen was pipetted into the assigned well.
3. 0.050ml (50 $\mu$ l) of the working Testosterone Enzyme Reagent was added to all wells .

4. The microplate was swirled gently for 30 seconds to mix.
5. 0.050ml (50 $\mu$ l) of Testosterone Biotin Reagent was to all wells.
6. The microplate was swirled gently for 30 seconds to mix.
7. Covered and incubated for 60 minutes at room temperature in dark environment.
8. The contents of the microplate were discarded by aspiration.
9. 350 $\mu$ l of wash buffer was added and aspirated, repeated two additional times for a total of three (3) washes. An automatic washer was used.
10. 0.100ml (100 $\mu$ l) of Working Substrate Solution was added to all wells. Reagents were added without shake in the same order to minimize reaction time differences between wells.
11. Incubated at room temperature for fifteen (15) minutes in dark environment.
12. 0.050ml (50 $\mu$ l) of stop solution was added to each well and gently mixed for 15- 20 seconds.
13. The absorbance in each well was read at 450nm within 30 minutes in a microplate reader.

#### **3.7.3.4 Quality control and calculation of the results:**

Reagents, Standard and control were checked for storage, stability and preparation before starting work. Each microwell plate was considered separately when the results were calculated and interrelated. In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 0ng/ml should be >1.3.
2. Four out of six quality control pools should be within the established ranges.

#### **3.7.3.5 Interpretation of results:**

Computer controlled data reduction was used to interpret the results of the test in spectrophotometer.

#### **3.7.3.6 Reference values:**

Low: < 2.5 ng/ml

Normal: 2.5-10 ng/ml



High: >10 ng/ml

#### **3.7.4 Calculate BMI, WC and WHR:**

Anthropometrics values were registered in a special form: weight, height, hip and waist (WC) were measured to calculate the waist-to-hip ratio (WHR). The BMI was calculated by the formula  $\text{weight (kg)}/\text{height}^2 \text{ (m}^2\text{)}$ . According to the BMI, the subjects were grouped in three categories: group (A), 20 subjects with  $\text{BMI} < 24.9 \text{ kg/m}^2$ , group (B), 20 subjects with BMI between 25.0 and 29.9  $\text{kg/m}^2$ , and group (C), 20 subjects with  $\text{BMI} > 30.0 \text{ kg/m}^2$ , we considered central obesity when the WHR was greater than 0.9m and the WC greater than 102 cm.

#### **3.8 Data analysis:**

Data were analyzed and tabulated using statistical package for social sciences (SPSS) program version 21, unpaired sample test, a crosstabs and correlation were performed by using Chi2 test estimated difference by P. value. In addition to that calculation of the means for numerical variables

#### **3.9 Ethical consideration:**

Faculty of Post-Graduate studies ethical committee, approved this study.

# Chapter Four

## **Results**

## 4. Results

**Table (4.1 ):-The Correlation Between BMI, WC and WHR with Testosterone**

	<b>BMI</b>	<b>WC</b>	<b>WHR</b>
<b>Mean</b>	27.02	101.53	.9115
<b>SD</b>	6.601	19.101	.07921
<b>Testosterone Pearson Correlation</b>	- 0.787	- 0.717	- 0.580
<b>p. values</b>	0.000	0.000	0.000

**Table (4.2): Means and standard deviations of Testosterone levels with Body mass Index (BMI)**

<b>BMI</b>	<b>NO</b>	<b>Mean</b>	<b>SD</b>	<b>P. value</b>
<b>Normal</b>	20	7.34	2.57	0.000
<b>Over Weight</b>	20	3.62	2.05	
<b>Obese</b>	20	2.08	0.58	

\* P value is significant

\* P value  $\leq 0,000$  significant

\*Normal BMI  $\leq 20$

\* Over Weight BMI  $>25$

\* Obese BMI  $>30$

**Table (4.3) The mean and standard deviations of Testosterone levels with waist Circumference**

<b>WC Level</b>	<b>No</b>	<b>Mean</b>	<b>SD</b>	<b>P. value</b>
<b>Normal</b>	32	5.85	3.21	0.001
<b>High</b>	28	3.03	1.89	

\* P value is significant

\*Normal (WC):-  $\leq 102$  /cm

\*High (WC):-  $>102$  /cm

**Table (4.4): The mean and standard deviations of Testosterone levels with Waist-Height Ratio**

<b>WHR Level</b>	<b>NO</b>	<b>Mean of Testosterone</b>	<b>SD</b>	<b>P value</b>
<b>Normal</b>	32	5.43	$\pm 3.52$	0.045
<b>High</b>	28	3.51	$\pm 1.89$	

\* P value is significant

\*Normal (WHR):-  $\leq 0.9$

\*High (WHR) :-  $> 0.9$

**Table (4.5 ): The mean and standard deviations of Testosterone levels with age.**

<b>Age</b>	<b>N</b>	<b>Mean</b>	<b>Std. Deviation</b>	<b>P.Value</b>
<b>18-28</b>	27	5.048	3.2570	0.061
<b>29-39</b>	18	4.994	3.1791	
<b>40-49</b>	15	2.943	1.3903	
<b>Total</b>	60	4.506	2.9789	

\* P value is insignificant .

**Table (4.6): The mean and standard deviations of Testosterone levels with Social status**

<b>Social status</b>	<b>No</b>	<b>Mean</b>	<b>SD</b>	<b>P .value</b>
<b>Single</b>	30	5.83	± 3.35	0.000
<b>Married</b>	30	3.18	± 1.79	

\* P value is significant (anova)

**Table (4.7): The mean and standard deviations of Testosterone levels in Smokers and non smokers.**

<b>Smoker</b>	<b>No</b>	<b>Mean</b>	<b>SD</b>	<b>P. value</b>
<b>Non smoker</b>	36	4.12	±2.59	0.017
<b>Smoker</b>	24	5.09	±3.47	

\* P value is significant (anova)

**Table (4.8): The effect of Exercise on Testosterone levels**

<b>Exercise</b>	<b>N</b>	<b>Mean</b>	<b>S. D</b>	<b>P.Value</b>
<b>Never</b>	27	4.53	3.57	0.474
<b>Regular</b>	24	4.90	2.68	
<b>Irregular</b>	9	3.44	1.34	
<b>Total</b>	60	4.51	2.98	

\* P value is Insignificant (anova).

**Table (4.9): The mean and standard deviations of Testosterone levels with Family History of Obesity.**

<b>History of Obesity</b>	<b>No</b>	<b>Mean</b>	<b>SD</b>	<b>P. value</b>
<b>FM of Obesity</b>	27	3.00	±1.84	0.000
<b>NO FM of Obesity</b>	33	5.73	±3.20	

\* P value is significant

# Chapter Five

**Discussion**

**Conclusion**

**Recommendations**



## 5.1 Discussion

In present study of testosterone levels among middle aged apparently healthy men with deferent ages and weights (normal, overweight and obese). Enrolled in Shendi locality was evaluated using Enzyme Linked Immunosorbent Assay (ELISA). Low serum testosterone were defined as total testosterone < 2.5 ng/ml, normal testosterone as 2.5-10 ng/ml.

Out of 60 blood specimens measured , the mean and SD testosterone levels in deferent weight (normal, overweight and obese) were (7.34± 2.57, 3.62 ± 2.05, 2.08 ± 0.58) respectively and the results showed 20/60(33.3%) had normal testosterone levels, 66.7% (overweight and obese) low testosterone levels, which was statistically highly significant (P value <0.000).

Significant inverse correlation of serum total testosterone with BMI( $r = - 0.787$ , P value = 0.000) , WC( $r = - 0.717$ , P value = 0.000) and WHR ( $r = - 0.580$ , P value = 0.000) was recorded in this study.

Results of present study were agreed partially with study done by Shamim MO-in 2015 : Statistically significant differences were observed in the mean values of BMI and WHR for the two groups of testosterone. Significant of serum total testosterone with BMI ( $r = -0.311$ , P value = 0.000) was recorded in this study. However testosterone was not significantly correlated with waist/hip ratio.( $r = 0.126$ , P value = 0.076) Middle age men working at DUHS who have low level of serum total testosterone are more obese than individuals with normal total testosterone level.<sup>(29)</sup> and also agreed with study done by (J. A. Osuna C. and R. G\_omez-Pe´ rez-2006) : relationship between low total and free testosterone concentrations with increased insulin resistance in obese men, they found that T and SHBG serum concentrations decreased as the BMI increased.<sup>(22)</sup>

The present finding WC of Normal and high the mean was (5.85± 3.21, 3.03± 1.89) respectively, WHR of normal and high the mean (5.43± 3.52, 3.51± 1.89)

respectively, Significant decreased of serum total testosterone with increased of WC (P value = 0.001) and WHR was (P value = 0.045).

Results of present study were insignificant relationship between age and serum total testosterone levels, the mean ages of deferent groups of age were mean and SD (5.05 , $\pm$ 3.30) (4.99,  $\pm$ 3.17), (2.94, $\pm$ 1.39) respectively, with (P value = 0.061).

Also study showed statistically significant effect on serum total testosterone levels with social status single and married where the mean was (5.81 $\pm$ 3.35), (3.18 $\pm$ 1.79) respectively with (P value =0.000).

Our study showed revealed statistically significant decreased of serum total testosterone levels with smoking were mean and SD of smokers and non smokers (5.09,  $\pm$ 3.47), (4.12, $\pm$ 2.59) respectively, with p. value ( 0.017 ).

In this study there was no statistically significant effect on serum total testosterone levels with Exercise were mean of group(never, regular, irregular) of exercise (4.53 $\pm$ 3.57),(4.90 $\pm$ 2.68), (3.44 $\pm$ 1.34) respectively with P value (0.474).

## 5.2 Conclusion

By the end of this study, we concluded that:

- The current study revealed statistically significant inverse correlation between serum total testosterone with BMI, WC and WHR .
- Also study showed no statistically significant relationship between age and serum total testosterone levels.
- The present finding lower serum total testosterone levels in married compared to single .
- The study revealed statistically significant decreased of serum total testosterone levels with smoking.
- No statistically significant effect on serum total testosterone levels with Exercise .

### **5.3 Recommendations**

- Weight loss can lead to increased testosterone level
- Smoking must be restricted
- Obese people must be on regular exercise to reduce their body weight
- Other study must be done with increase sample size with deferent parameters( SHBG,free and total testosterone level ) .

# Chapter six

**References**

**Appendixes**

## 6.1 References

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## 6.2 Appendixes

### Appendix (1): Clinical Evaluation Form (Questionnaire)

#### Shendi University

Faculty of Post-Graduate studies

### Association Between Obesity and Serum Total Testosterone in Middle Aged Healthy Men

#### *Questionnaire*

#### Personal information:

Index NO  Age:-

#### Social Status:-

Single  Married  Divorced

#### Social habits

Do you smoke? Yes  No

Do you engage exercise?

Regular  Irregular  Never

#### Chemical analysis:

Testosterone: ng/dl

BMI :

(WC) :  c/m

WHR:

**Appendix (2):** Photometric measurement for detection of ELISA cooler density.



**Appendix (3):** Microtitre showing antibody reactivity for Testosterone -Ab.

