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Evaluation of Testosterone Hormone Level in Healthy Obese Subjects in Khartoum State

تقويم مستوى هرمون التستوسترون لدى الأشخاص الأصحاء الذين يعانون من السمنة في ولاية الخرطوم

A dissertation submitted in a partial fulfillment for the requirement of M.Sc degree in clinical chemistry

By

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Dedication

I would like to dedicate this study to fountain of love and tenderness

To my mother

To the source of inspiration and strength

To my father

To the one I love

To all my family, friends and colleagues

Acknowledgments

I would like to give appreciated thanks to Allah.

And I’m very grateful to my supervisor Dr.Ghada Abdelrahman Elfadil for her patience, support and continuous guidance.

To all my family, friends, collages, patients who supported and helped me to complete this study.

And special thanks to Reproductive health care centre.

All thanks to ever who lent a helping hand to me.

Abstract

Back ground: Obesity is a state of excess adipose tissue, associated with abnormal sex steroids concentrations which are related to the degree of obesity. This study was carried out to assess serum level of testosterone hormone in obese subjects.

Material and methods: fifty samples were collected from obese and overweight (male and female) in period between August to November 2017, chosen randomly in Khartoum State, and 50 apparently healthy individuals with normal BMI as control group, to assess the effect of obesity on testosterone hormone level. Estimation of serum testosterone level was done by using enzyme immune assay analyzer (TOSOH), and results were analyzed using Statistical Package of Social Science (SPSS) computer program.

Result: The result of this study showed that the mean concentration of testosterone hormone in obese subjects was significantly decreased compared to control (Mean ± SD for cases versus control). (Mean ± SD: 4.25±1.97 versus 9.97±10.74, *P. value*=0.003). It showed that the mean concentration of testosterone in obese male was significantly decreased compared to control male (4.17 ± 1.05 versus 22.05 ± 6.34, *P.value* =0.00). In addition it showed that mean concentration of testosterone in obese female was significantly increased compared to control (4.70±2.97 versus 1.92 ± 0.89, *P.value* =0.00). Also showed that mean concentration of testosterone in obese class 1 was significantly increased compared to control (3.95±1.89 versus 9.97±10.74, *P. value*=0.008). It showed strong positive correlation between BMI and Testosterone in obese female(r=0.5, p=0.03).

Conclusion: It is concluded that the serum level of testosterone hormone decreases in Sudanese obese male but increases in obese female.

المستخلص

خلفية: السمنة متعلقة بتغييرات غير طبيعية في مستويات هرمونات الخصوبة الاسترويدية والتي لها علاقة حسب درجة السمنة.

الهدف من الدراسة: تقويم مستوى هرمون التستوسترون لدى الأشخاص الذين يعانون من السمنة.

الأدوات والطريقة: تم جمع 50 عينة من الأشخاص الذين يعانون من السمنة و زيادة الوزن في الفترة من أغسطس حتى نوفمبر, أختيرت عشوائيا في ولاية الخرطوم. و50 من الأفراد الأصحاء كمجموعة ضابطة، لتقييم تأثير السمنة على مستوى التستسوتسترون في مصل الدم.تم قياس مستوى هرمون التستوسترون بإستخدام جهاز Tosoh، وتم تحليل النتائج باستخدام برنامج نظام الحزمة الإحصائية للعلوم الاجتماعية (SPSS)، برنامج الكمبيوتر.

النتيجة :أظهرت نتائج الدراسة ان متوسط تركيز هرمون التستوسترون منخفض بشكل ملحوظ لدى الاشخاص الذين يعانون من السمنة مقارنة بمجموعة التحكم."المتوسط ± الإنحراف المعياري للمرضى مقارنة بمجموعة التحكم"(1.97±4.25مقابل 10.74±9.97 ، وكان الاحتمال الاحصائي للمقارنة 0.003).

كما أظهرت الدراسة أن متوسط تركيز التستوسترون لدى الرجال الذين يعانون من السمنة منخفض بشكل ذو دلالة إحصائية بالمقارنة بمجموعة التحكم (1.05± 4.17 مقابل 6.34±22.05 ,و كان الإحتمال الإحصائي0.00).

إضافة إلى ذلك أظهرت الدراسة أن متوسط تركيز التستوسترون لدى النساء الذين يعانين من السمنة مرتفع بشكل ذو دلالة إحصائية بالمقارنة بمجموعة التحكم (2.97±4.70 مقابل 1.92 , 0.89 ± و كان الإحتمال الإحصائي0.00). كما أن متوسط تركيز التستوسترون لدى الأشخاص الذين يعانون من السمنة الفئة الأولى لديهم زيادة ذات دلالة احصائية مقارنة بمجموعة التحكم (1.89±3.95مقابل 10.74±9.97وكان الاحتمال الاحصائي0.008).

الخلاصة :خلصت الدراسة الى أن مستوى تركيز هرمون التستوتسترن يقل بشكل ملحوظ لدى الرجال الذين يعانون من السمنة بينما يزيد في النساء.

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List of abbreviations

Abbreviation	Full Terms
ABPs	Androgen binding proteins
ACTH	Adrenocortico tropic hormome
AMH	Anti-mullerian hormone
APVP	Alpha pyrrolidinovalerophenone
ARC	Activity regulated cytoskeleton
BMI	Body Mass Index
CAH	Congenital adrenal hyperplasia
CHD	Coronary Heart Disease
CRH	Corticotropin releasing hormone
CT	Computed tomography
DHEA	Dehydroepiandrosterone
DHT	Dehydrotestosterone
FSH	Follicle stimulating hormone
FSH	Follicle stimulating hormone beta
GABA	Gamma aminobutyric acid
GH	Growth hormone
GLP	Glucagon like receptor
GnIH	Gonadotropin inhibitory hormone
GnRH	Gonadotropin releasing hormone
GnRH 1	Gonadotropin releasing hormone
HCG	Human chorionic gonadotropin
ICSH	Interstitial cell–stimulating hormone
IGF	Insuln like growth factor

IU	International units
KP	Kisspeptin
LC-MS	Liquid chromatograpgh-mass spectrometry
LH	Luetinizing hormone
MAO	Monoamine oxidase
MC4R	Melanocortin4 receptor
MIH	Mullerrian inhibiting hormone
MRI	Magnetic resonance imaging
NASH	Non alcoholicsteatohepatits
NCBI	National center for biotechnology information
NHLBI	National heart, lung and blood institue
NIEHS	National institute of environmental health science
PCOS	Poly cystic ovarian syndrome
PID	Pelvic inflammatory disease
SHBG	Sex hormone binding globulin
T2DM	Type2 diabetes mellitus
TSH	Thyroid stimulating hormone
WHO	World Health Organization
WHR	Waist hip ratio

Chapter One

Introduction, Rationale and Objectives

1. Introduction, Rationale and Objectives

1.1 Introduction

Obesity is a state of excess adipose tissue mass; it is effectively defined by assessing its linkage to morbidity or mortality (Jameson., 2010).

It is defined by the World Health Organization as a BMI of > 30 kg/m2. The condition is associated with increased mortality, not only from cardiovascular disease and diabetes mellitus but also from cancer. Obesity also significantly increases morbidity from many associated conditions, such as arthritis and sleep apnoea (Fox *et al.*, 2015).

Obesity is becoming a global epidemic. In the UK, a quarter of adults are obese and more than half are overweight (or obese). In the USA this increases to 39% obese and 69% overweight (or obese). This places a huge burden on medical resources (Fox *et al.*, 2015).

Disorders that affect the reproductive axis are associated with obesity in both men and women. Male hypogonadism is associated with increased adipose tissue, often distributed in a pattern more typical of females. In men >160% ideal body weight, plasma testosterone and sex hormone–binding globulin (SHBG) are often reduced, and estrogen levels (derived from conversion of adrenal androgens in adipose tissue) are increased. Gynecomastia may be seen. However, masculinization, libido, potency, and spermatogenesis are preserved in most of these individuals. Free testosterone may be decreased in morbidly obese men whose weight is >200% ideal body weight. Obesity has long been associated with menstrual abnormalities in women, particularly in women with upper body obesity. Common findings are increased androgen production, decreased SHBG, and increased peripheral conversion of androgen to estrogen. Most obese women with

oligomenorrhea have the polycystic ovarian syndrome (PCOS), with its associated anovulation and ovarian hyperandrogenism; 40% of women with PCOS are obese. In obese women with PCOS, weight loss or treatment with insulin-sensitizing drugs often restores normal menses. The increased conversion of androstenedione to estrogen, which occurs to a greater degree in women with lower body obesity, may contribute to the increased incidence of uterine cancer in postmenopausal women with obesity (Jameson., 2010).

1.2 Rationale

The worldwide prevalence of obesity has increased dramatically over the last several decades (Larsen *et al.*, 2003), and excess weight has been associated with increased risk of cancer, which modulated through alterations in the metabolism of sex steroid hormones and related peptides (Lukanova *et al.*, 2004).

So the study aims to demonstrate the relation between testosterone hormone level and obesity.

And there are no published studies about this in Sudan, so it will help to provide knowledgement and monitoring of testosterone hormones in obese subjects.

1.3 Objectives:

1.3.1 General Objective:

To evaluate the level of testosterone hormone in obese Sudanese patients in Khartoum state.

1.3.2 Specific Objectives:

1- To estimate testosterone hormone level in study group and control group.

2- To correlate between testosterone hormone level with BMI in cases.

3- To compare between testosterone hormone and sex.

4-To correlate testosterone hormone level with obesity.

Chapter Two

Literature review

2. Literature Review

2.1 Obesity

Obesity is a chronic disease (Reed *et al.*, 2003) defined as BMI of > 30 kg/m2, a measurement obtained by dividing a person's weight by the square of the person's height (WHO., 2015).The condition is associated with increased mortality, not only from cardiovascular disease and diabetes mellitus but also from cancer. Obesity also significantly increases morbidity from many associated conditions, such as arthritis and sleep apnoea (Fox *et al.*, 2015)

Worldwide 600 million people are thought to be obese, accounting for around 15% of the world's population. In the UK, approximately 25% of the adult population are obese, with a further 35% classed as overweight. Obesity rates are continuing to rise, such that obesity is estimated to affect 60% of adult men, 50% of adultwomen and 25% of children in the UK by 2050 (Rees *et al.*, 2017).

2.1.1 Causes of Obesity

Chronic excess of nutrient intake relative to the level of energy expenditure (Jameson., 2010).

Overweight and obesity results from a complex interaction between environmental pressures and risks and genetic susceptibility (Wass *et al.*, 2014), summarized as follow:

I. Genetic Factors

Monogenic obesity, mutations in genes (usually related to appetite control within the hypothalamus) are associated with obesity of early childhood onset, usually with hyperphagia. However, only about 5% of all severe childhood and 2% of adult obesity are associated with identified genetic

causes. Of these, mutations in the melanocortin 4 receptor (MC4R) are the most frequent and are associated with increased linear growth, fat and lean mass, hyperphagia (moderate) and severe hyperinsulinaemia, but normal puberty and fertility. An increasing number of genes associated with the development of obesity have been identified through genome-wide association studies (Wass *et al.,* 2014).

Evidence for genetic causes of obesity was initially provided by the occurrence of familial obesity, and scientific evidence by the observation of massive obesity in mutant ob/ob mice with a recessively inherited disease. The mice eat voraciously and develop symptoms of Type 2 diabetes. Apart from their hyperlipidaemia, hyperphagia, hyperglycaemia and insulin resistance, the mice are also hypothermic and infertile. The ob gene was cloned and its product expressed and termed leptin (Greek leptos, which means thin). It is expressed only by fat cells (Greenstein and wood., 2011).

II. Environmental Factors

The drivers of obesity can be considered under two main headings: increased energy (food) intake or decreased energy expenditure due to physical inactivity, Societal changes, e.g. increased availability of high caloric density foods and sedentary lifestyle, have been one of the main causes of changes in this balance (Greenstein and wood., 2011).

III. Secondary Causes

- Hypothyroidism

The possibility of hypothyroidism should be considered, but it is an uncommon cause of obesity; hypothyroidism is easily ruled out by measuring thyroid-stimulating hormone (TSH). Much of the weight gain that occurs in hypothyroidism is due to myxedema (Jameson., 2010).

❖ Cushing's disease/syndrome

It reflects the symptoms produced by excess cortisol secretion in to the circulation, although the obesity produced is due to redistribution of fat to the face, neck and abdominal region. There is also significant fluid retention with attendant cardiovascular problems due to the mineralocorticoid action of cortisol when present in the blood in high concentrations (Greenstein and wood., 2011).

Although obese patients commonly have central obesity, hypertension, and glucose intolerance, they lack other specific stigmata of Cushing's syndrome. Nonetheless, a potential diagnosis of Cushing's syndrome is often entertained. Cortisol production and urinary metabolites (17OH steroids) may be increased in simple obesity. Unlike in Cushing's syndrome, however, cortisol levels in blood and urine in the basal state and in response to corticotropin-releasing hormone (CRH) or ACTH are normal; the overnight 1-mg dexamethasone suppression test is normal in 90%, with the remainder being normal on a standard 2-day low-dose dexamethasone suppression test. Obesity may be associated with excessive local reactivation of cortisol in fat by 11β-hydroxysteroid dehydrogenase 1, an enzyme that converts inactive cortisone to cortisol (Jameson., 2010).

❖ Hypothalamic lesions:

Hypothalamic dysfunction of systems controlling satiety, hunger and energy expenditure can cause varying degrees of obesity. It is uncommon to identify a discrete anatomic basis for these disorders. Subtle hypothalamic dysfunction is probably a more common cause of obesity that can be documented using currently available imaging techniques. Growth hormone (GH), which exerts lipolytic activity, is diminished in obesity and is increased with weight loss. Despite low GH levels, insulin-like growth

factor (IGF) I (somatomedin) production is normal, suggesting that GH suppression is a compensatory response to increased nutritional supply (Jameson., 2010).

- Polycystic ovary syndrome (PCOS)

Strongly linked to overweight and obesity but mechanism unclear (Wass *et al.,* 2014). Obesity is found in around 50% of women with polycystic ovary syndrome (PCOS). Furthermore, lean women with PCOS demonstrate lesser degrees of hyperinsulinaemia and insulin resistance, which play a role in the pathogenesis of PCOS independently of obesity as insulin stimulates ovarian androgen production. The metabolic consequences of obesity and hyperinsulinaemia are seen in women with PCOS who have a high risk of developing impaired glucose tolerance and Type 2 diabetes. Clinical evidence of hyperinsulinaemia may be seen as acanthosisnigricans, a brown velvety pigmentation usually seen at the base of the neck and in the axillae in obese women with PCOS (Greenstein and wood., 2011).

- Insulinoma

Patients with insulinoma often gain weight as a result of overeating to avoid hypoglycemic symptoms. The increased substrate plus high insulin levels promote energy storage in fat. This can be marked in some individuals but is modest in most (Jameson., 2010).

- Iatrogenic

Drugs, e.g. antipsychotic medication, hypoglycaemics (including insulin) glucocorticoids, And recreational drugs, e.g. cannabis (Wass *et al*., 2014).

2.1.2 Clinical Features of Obesity:

Patients who are obese have certain clinical features:

- ❖ Apple' body shape with increased abdominal girth, or a 'pear' body shape, which has increased deposition of fat around the gluteal region and upper leg girth
- ❖ Signs of insulin resistance: skin tags, acanthosisnigricans, hirsutism (in women)
- ❖ Signs of organ failure: cardiomegaly, respiratory compromise, tender hepatomegaly from nonalcoholic steatohepatitis (NASH)

Signs of complications: candida infections, pressure ulceration, osteoarthritis (Fox *et al.*, 2015).

2.1.3 Pathophysiology of Obesity

Energy balance and body weight are regulated, but the main drive of this allostatic physiology is towards energy acquisition (and thus fat deposition) and defence against weight loss. Although physiology can be 'overridden' by cognitive and behavioural control (e.g. diet, exercise) long-term, these mechanisms (within our obesogenic environment) usually prove insufficient in the long-term either to protect against or reverse weight gain. While leptin and ghrelin are produced peripherally, they control appetite through their actions on the central nervous system, they and other appetite-related hormones act on the hypothalamus (Files., 2004).

In human obesity, leptin levels are high, rather than low, correlating with fat mass, suggesting either that leptin 'resistance' is present or that leptin is a starvation, rather than obesity signal (Wass *et al.*, 2014).

Gut peptide hormones released after food intake provide acute signals of hunger, satiety, and fullness. Although originally thought to be short-term signals, the importance of the gut–brain axis as a regulator of body weight in humans has become increasingly apparent from the effects of bariatric surgery. Ghrelin, a hunger hormone, rises before, and probably is involved

with, initiation of food intake. Satiety hormones released after food include glucagon-like peptide-1 (GLP-1), an incretin hormone secreted by ileal L-cells in the distal intestine that stimulates insulin secretion, and peptide YY secreted from the ileum and colon. Oxyntomodulin (also derived from preproglucagon) reduces food intake and increases energy expenditure after systemic administration. Weight is gained or lost usually in the proportion of 70% fat and 30% lean tissue, implying that approximately 30MJ (7,000kcal) surplus or deficit is needed to gain or lose 1 kg in body mass. During the first days of a very low energy or ketogenic diet, liver glycogen may be the primary source of stored energy to meet metabolic needs; since it provides about 8MJ/kg, initial weight loss is more rapid than with less severe energy restriction (Wass *et al.*, 2014).

2.1.4 Complications of Obesity

Excessive body weight is associated with various diseases and conditions, particularly cardiovascular diseases, diabetes mellitus type 2, obstructive sleep apnea, certain types of cancer, osteoarthritis and asthma. As a Effects on health result, obesity has been found to reduce life expectancy (Hashlam and James., 2005).

2.1.5 Mortality of Obesity

Obesity is one of the leading preventable causes of death worldwide (Barness *et al.*, 2007).A number of reviews have found that mortality risk is lowest at a BMI of 20– 25 kg/m2 (Aune *et al.*, 2016) in non-smokers and at relative risk of death over 10 years for white men and women who have never smoked in the United States by BMI (Berrington., 2010) 24–27 kg/m2 in current smokers, with risk increasing along with changes in either direction (Pischon *et al.*, 2008) This appears to apply in at least four continents In contrast, a 2013 review found that grade 1 obesity (BMI 30-35)

was not associated with higher mortality than normal weight, and that overweight (BMI 25-30) was associated with "lower" mortality than was normal weight (BMI 18.5-25) (Flegal *et a.,* 2013) Other evidence suggests that the association of BMI and waist circumference with mortality is U- or J shaped, while the association between waist-to-hip ratio and waist-to-height ratio with mortality is more positive (Carmienke *et al.,* 2013).

2.1.6 Diagnostic approach of Obesity

- **BMI and Waist Circumference**

Three key anthropometric measurements are important to evaluate the degree of obesity: weight, height, and waist circumference. BMI is used since it provides an estimate of body fat and is related to risk of disease. Excess abdominal fat, assessed by measurement of waist circumference or waist-to-hip ratio, is independently associated with higher risk for diabetes mellitus andcardiovascular disease. Measurement of the waist circumference is a surrogate for visceral adipose tissue and should be performed in the horizontal plane above the iliac crest (Jameson., 2010).Waist circumference predicts risk of metabolic complications, increased risk in men ≥94 cm and ≥88 cm in women. Waist circumference correlates with measures of risk for CHD such as hypertension or blood lipid levels (Peter., 2000).

The use of BMI as a measure of relative adiposity has been documented in a number of studies, the rate of mortality associated with BMI is generally higher for lower and higher BMI values and lower for moderate levels of BMI. This curve, generally termed a U-shape curve (Renato and Valentina., 2001).

Body mass index (BMI) is calculated by dividing weight (in kilograms) by height (in meters squared) or by dividing weight (in pounds) multiplied by 704 by height (in inches squared) (Larsen *et al.*, 2003).

Weight classification by BMI: according to the World Health Organization's (WHO) classification system (WHO., 2017).

Table (2-1): WHO Classification of Obesity:

Weight classification	BMI (kg/m^2)
Underweight	< 18.5
Normal	18.5 – 24.9
Overweight	25 – 29.9
Obese: class 1	30 – 34.9
Obese: class 2	35 -39.9
Obese: class 3	≥ 40

Any BMI ≥ 35 or 40 kg/m2 is severe obesity, a BMI of ≥ 35 kg/m2 and experiencing obesity-related health conditions or ≥40–44.9 kg/m2 is morbid obesity, a BMI of ≥ 45 or 50 kg/m2 is super obesity (Kanazawa *et al.,* 2005). BMI does not provide information about the composition or distribution of weight, and cannot distinguish between muscle, bone and fat. These limitations can cause problems such as:

- Overestimation of body fat in patients who gain muscle and lose fat, but do not change weight.
- Underestimation of body fat in older patients because lean body mass gradually declines with age.
- Underestimation of body fat in South Asians (Green., 2009).

Although not a direct measure of adiposity, the most widely used method to gauge obesity. Other approaches to quantify obesity include anthropometry (skin fold thickness), densitometry (underwater weighing), CT or MRI, and electrical impedance (Jameson., 2010).

- **Waist–hip Ratio (WHR)**

Is the dimensionless ratio of the circumference of the waist to that of the hips. This is calculated as waist (H) measurement divided by hip(H) (W ÷ H).The WHR has been used as an indicator or measure of health, and the risk of developing serious health conditions. (Morkedal *et al.,* 2011)

The waist circumference should be measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest, using a stretch resistant tape that provides a constant 100 g tension. Hip circumference should be measured around the widest portion of the buttocks, with the tape parallel to the floor, two measurements should be repeated (WHO., 2008).

WHR has been found to be a more efficient predictor of mortality in older people (>75 years of age) than waist circumference or BMI (Price *et al.*, 2006).WHR has been shown to be a better predictor of cardiovascular disease than waist circumference and body-mass index (Morkedal *et al.*, 2011).WHR correlates with fertility (with different optimal values in males and females). A WHR of 0.9 for men and 0.7 for women has been shown to correlate strongly with general health and fertility. Women within the 0.7 range have optimal levels of estrogen and are less susceptible to major diseases such as diabetes, cardiovascular disorders and ovarian cancers Women with high WHR (0.80 or higher) have significantly lower pregnancy rates than women with lower WHRs (0.70–0.79), independent of their BMIs (singh., 2002).

2.1.7 Management of Obesity

Managing obesity presents a huge challenge for the global healthcare community. Prevention is crucial but public health campaigns to date have failed to impact significantly onthis growing epidemic. Patients with

established obesity should target at least a 10% weight loss as this is associated with significant reduction in morbidity and mortality. Dietary strategies aimed at reducing energy intake should be used, in addition to increasing the amount of physical activity (Rees *et al.*, 2017).

2.1.8 Treatment of Obesity

Obesity is a chronic disorder associated with significant morbidity, impaired quality of life and increased mortality rates. Treatment of obesity is difficult, not only due to the need for obese individuals to make significant lifestyle changes, but also due to prejudices held by society and doctors towards the condition and its management. The principle of treating obesity is simple, to produce a negative energy balance that utilizes body stores and is maintained in the long term. The practice is more complex, requiring education about diet and activity levels and, where deemed necessary, the introduction of pharmacological agents in addition to lifestyle modification (Greenstein and wood., 2011).

2.1.8.1 Drug therapies of obesity

Drug therapies available to treat obesity are limited. Orlistat, an inhibitor of pancreatic and gastric lipases, can result in a modest reduction in weight of up to 10%. However, treatment is often poorly tolerated as a result of steatorrhoea from fatvmalabsorption, GLP-1 receptor agonists may have a future role as they are currently known to induce significant weight loss in many patients with T2DM. (Aled *et al.*, 2017). Such as neuropeptide Yantagonists (Greenstein and wood., 2011).

2.1.8.2 Lifestyle management of obesity

Obesity care involves attention to three essential elements of lifestyle: dietary habits, physical activity, and behavior modification. Because obesity is fundamentally a disease of energy imbalance, all patients must learn how

and when energy is consumed (diet), how and when energy is expended (physical activity), and how to incorporate this information into their daily life (behavior therapy). Lifestyle management has been shown to result in a modest (typically 3–5 kg) weight loss compared to no treatment or usual care (Jameson., 2010).

- **Diet Therapy of obesity**

The primary focus of diet therapy is to reduce overall calorie consumption. The NHLBI guidelines recommend initiating treatment with a calorie deficit of 500–1000 kcal/d compared to the patient's habitual diet. This reduction is consistent with a goal of losing approximately 1–2 lb per week. This calorie deficit can be accomplished by suggesting substitutions or alternatives to the diet (Jameson., 2010).

- **Physical Activity Therapy**

Although exercise alone is only moderately effective for weight loss, the combination of dietary modification and exercise is the most effective behavioral approach for the treatment of obesity (Jameson., 2010).

- **Behavioral Therapy**

Cognitive behavioral therapy is used to help change and reinforce new dietary and physical activity behaviors. Strategies include self monitoring techniques (e.g., journaling, weighing, and measuring food and activity), stress management, stimulus control (e.g., using smaller plates, not eating in front of the television or in the car),social support, problem solving, and cognitive restructuring to help patients develop more positive and realistic thoughts about themselves. When recommending any behavioral lifestyle change, have the patient identify what, when, where and how the behavioral change will be performed. The patient should keep a record of the anticipated behavioral change so that progress can be reviewed at the next

office visit. Because these techniques are time-consuming to implement, they are often provided by ancillary office staff such as a nurse clinician or registered dietitian (Jameson., 2010).

2.1.8.3 Surgery of obesity

Surgical therapy such as gastric banding to reduce gastric size remains an option for patients with morbid obesity who have failed dietary and medical interventions and is the most effective treatment for individuals with a BMI > 40 kg/ m^2 (Greenstein and wood., 2011).Currently, bariatric surgery remains the only treatment shown to reduce weight significantly in the long term. Restrictive (gastric banding or sleeve gastrectomy) or malabsorptive (gastric bypass) procedures can be undertaken, but surgery in the UK is currently restricted to patients with a BMI of 40 kg/m2 or more, or 35–40 kg/m2 if significant co-morbidity (e.g. T2DM or hypertension) potentially amenable to improvement with weight loss is present. All other non-surgical measures must have been tried first. Bariatric surgery is associated with a resolution of newly established T2DM in up to 80% of cases, hence patients with a recent diagnosis of T2DM and BMI ≥35 kg/m² can be assessed for surgery (Rees *et al.*, 2017).

2.1.9 Prognosis of obesity

It is estimated that 25% of the ischaemic heart disease burden, 45% of the diabetes burden and up to 40% of certain cancers are caused by overweight and obesity. At least 2.8 million adults die each year as a result of being overweight or obese (Rees *et al*, 2017), obesity reduces expectancy (Hashlam and James., 2005).

2.2 Human Reproductive System

The female reproductive system is made up of the internal and external sex organs that function in human reproduction. The female reproductive system

is immature at birth and develops to maturity at puberty to be able to produce gametes, and to carry a fetus to full term. (Mahadevan *et al.,* 2013).

Normal adult male testicular volume15–30 mL and normal adult female ovarian volume 5–10 mL, two gonadotrophins LH and FSH address two gonadal cell types, with feedback from sex steroids and inhibin (Wass *et al.*, 2014).

Three important cells of the gonad:

- ❖ Interstitial cells: male Leydig cells, female theca cells, which are found in between the seminiferous tubules and follicles, respectively. Produce testosterone under LH drive (Wass *et al.,* 2014).
- ❖ Cells supporting gametogenesis: male Sertoli cells, female granulosa cells, secrete various hormones, including inhibin and Müllerian inhibitory factor (AMH) under FSH drive. The former inhibits FSH secretion from the pituitary gland, and the latter is responsible for suppressing female sex organ development during sexual differentiation in utero. In adult male, AMH is produced in proportion to germ cell number and is, therefore, a marker of ovarian ageing.
- ❖ Germ cells: continue to make new germ cells throughout adult life in the basal membrane of tubules. Male cease to make new germ cells after birth and are, therefore, born with all of the ‘eggs’ that they will ever make (Wass *et al.,* 2014).

- Male seminiferous tubules, Make up 90% of testicular volume. Spermatogenesis occurs here in the presence of high intratesticular concentrations of testosterone. Made up of germ cells and Sertoli cells through which spermatogonia mature to be released into the lumen of the tubule.

- Female graafian follicle, Primordial follicles are recruited in batches, mature over 2 months, with selection of a dominant follicle with single central oocyte surrounded by granulosa cells which convert theca-derived testosterone to oestradiol for ovulation. Follicles which do not proceed to ovulation become atretic (Wass *et al.*, 2014).

2.2.1 Gonad (Reproductive Gland)

Is a mixed gland that produces the gametes (sex cells) and sex hormones of an organism. The male gonad, the testicle, produces sperm in the form of spermatozoa. The female gonad, the ovary, produces egg cells. The gonads are controlled by luteinizing hormone and follicle-stimulating hormone, produced and secreted by gonadotropes in the anterior pituitary gland. This secretion is regulated by gonadotropin-releasing hormone produced in the hypothalamus (Marieb., 2013).

2.2.2 Sex hormones

I. Follicle-stimulating hormone (FSH) is a gonadotropin, a glycoprotein polypeptide hormone. FSH is synthesized and secreted by the gonadotropic cells of the anterior pituitary glandand regulates the development, growth, pubertal maturation, and reproductive processes of the body. FSH and luteinizing hormone (LH) work together in the reproductive system. The alpha subunits of the glycoproteins LH, FSH, TSH, and hCG are identical and consist of about 96 amino acids, while the beta subunits vary (NCBI., 2016) Both subunits are required for biological activity. FSH has a beta subunit of 111 amino acids (FSH β), which confers its specific biologic action, and is responsible for interaction with the structure follicle-stimulating hormone receptor (Jiang., 2012). In both males and females, FSH stimulates the maturation of germ cells. In males, FSH induces Sertoli cells to secrete androgen-binding proteins (ABPs), regulated by inhibin's

negative feedback mechanism on the anterior pituitary. Specifically, activation of Sertoli cells by FSH sustains spermatogenesis and stimulates inhibin B secretion. In females, FSH initiates follicular growth, specifically affecting granulosa cells. With the concomitant rise in inhibin B, FSH levels then decline in the late follicular phase. This seems to be critical in selecting only the most advanced follicle to proceed to ovulation. At the end of the luteal phase, there is a slight rise in FSH that seems to be of importance to start the next ovulatory cycle. Control of FSH release from the pituitary gland is unknown (Sharma., 2012). GnRH has been shown to play an important role in the secretion of FSH, with hypothalamic pituitary disconnection leading to a cessation of FSH. GnRH administration leads to a return of FSH secretion. FSH is subject to oestrogen feed-back from the gonads via the hypothalamic pituitary gonadal axis (Häggström., 2014).

Follicle stimulating hormone is typically measured in the early follicular phase of the menstrual cycle, typically day three to five, counted from last menstruation. At this time, the levels of estradiol (E2) and progesterone are at the lowest point of the menstrual cycle. FSH levels in this time is often called basal FSH levels, to distinguish from the increased levels when approaching ovulation. FSH is measured in International Units (IU). For Human Urinary FSH, one IU is defined as the amount of FSH that has an activity corresponding to 0.11388 mg of pure Human Urinary FSH. For recombinant FSH, one IU corresponds to approximately 0.065 to 0.075 µg of a "fillby-mass" product (Radu., 2010).

The most common reason for high serum FSH concentration is in a female who is undergoing or has recently undergone menopause. High levels of Follicle-Stimulating Hormone indicate Disease states that the normal restricting feedback from the gonad is absent, leading to an unrestricted

pituitary FSH production. If high FSH levels occur during the reproductive years, it is abnormal. Conditions with high FSH levels include: Premature menopause, Poor ovarian reserve also known as Premature Ovarian Aging, Gonadal dysgenesis, Turner syndrome, Castration, Swyer syndrome, Certain forms of CAH ,Testicular failure, Klinefelter syndrome, Systemic Lupus Erythematosus also known as Lupus, Most of these conditions are associated with subfertility and/or infertility. Therefore, high FSH levels are an indication of subfertility and/or infertility (Radu., 2010).

Diminished secretion of FSH can result in failure of gonadal function (hypogonadism). This condition is typically manifested in males as failure in production of normal numbers of sperm. In females, cessation of reproductive cycles is commonly observed. Conditions with very low FSH secretions are: Polycystic Ovarian Syndrome (Obesity, Hirsutism, Infertility), Kallmann syndrome, Hypothalamic suppression, Hypopituitarism, Hyperprolactinemia, Gonadotropin deficiency, Gonadal suppression therapy (Radu., 2010).

II. Luteinizing hormone (LH) is a hormone produced by gonadotropic cells in the anterior pituitary gland. In females, an acute rise of LH triggers ovulation and development of the corpus luteum. In males, where LH had also been called interstitial cell– stimulating hormone (ICSH), it stimulates Leydig cell production of testosterone. It acts synergistically with FSH (Jiang., 2014). LH is a heterodimeric glycoprotein. Each monomeric unit is a glycoprotein Luteinizing hormone beta polypeptide Structure molecule; one alpha and one beta subunit make the full, functional protein. Its structure is similar to that of the other glycoprotein hormones, (FSH), (TSH), (hCG). The protein dimer contains 2 glycopeptidic subunits, labeled alpha and beta subunits, that are non-covalently associated (Jiang., 2014).

LH supports theca cells in the ovaries that provide androgens and hormonal precursors for estradiol production. At the time of menstruation, FSH initiates follicular growth, specifically affecting granulosa cells. With the rise in estrogens, LH receptors are also expressed on the maturing follicle, which causes it to produce more estradiol. Eventually, when the follicle has fully matured, a spike in 17αhydroxyprogesterone production by the follicle inhibits the production of estrogens, leading to a decrease in estrogen-mediated negative feedback of GnRH in the hypothalamus, which then stimulates the release of LH from the anterior pituitary. However another theory of the LH peak is a positive feedback mechanism from estradiol. The levels keep rising through the follicular phase and when they reach an unknown threshold, these results in the peak of the LH. This effect is opposite from the usual negative feedback mechanism presented at lower levels. In other words, the mechanism(s) are not yet clear (Mahesh, 2012). The increase in LH production only lasts for 24 to 48 hours. This triggers ovulation, thereby not only releasing the egg from the follicle, but also initiating the conversion of the residual follicle into a corpus luteum that in turn, produces progesterone to prepare the endometrium for a possible implantation. LH is necessary to maintain luteal function for the second two weeks of the menstrual cycle. If pregnancy occurs, LH levels will decrease, and luteal function will instead be maintained by the action of hCG (human chorionic gonadotropin), a hormone very similar to LH but secreted from the new placenta. In males, LH acts upon the Leydig cells of the testis and is regulated by (GnRH).The Leydig cells produce testosterone under the control of LH, which regulates the expression of the enzyme 17βhydroxysteroid dehydrogenase that is used to convert androstenedione the hormone produced by the testes, to testosterone, an androgen that exerts

both endocrine activity and intratesticular activity on spermatogenesis. LH is released from the pituitary gland, and is controlled by pulses of gonadotropin-releasing hormone. When Testosterone levels are low, GnRH is released by the hypothalamus, stimulating the pituitary gland to release LH. As the levels of Testosterone increase, it will act on the hypothalamus and pituitary through a negative feedback loop and inhibit the release of GnRH and LH consequently. Androgens (T, DHT) inhibit monoamine oxidase (MAO) in pineal, leading to increased melatonin and reduced LH and FSH by melatonininduced increase of GnIH synthesis and secretion. T can also be aromatized into estradiol (E2) to inhibit LH. E2 decreases pulse amplitude and responsiveness to GnRH from the hypothalamus onto the pituitary (Pitteloud *et al*., 2008).

Gonadal steroids (estrogens and androgens) generally have negative feedback effects on GnRH-1 release at the level of the hypothalamus and at the gonadotropes, reducing their sensitivity to GnRH. Positive feedback by estrogens also occurs in the gonadal axis of female mammals and is responsible for the midcycle surge of LH that stimulates ovulation. Although estrogens inhibit kisspeptin (Kp) release from kiss1 neurons in the ARC, estrogens stimulate Kp release from the Kp neurons in the AVPV. As estrogens' levels gradually increase the positive effect predominates, leading to the LH surge. GABA-secreting neurons that innervate GnRH-1 neurons also can stimulate GnRH-1 release. These GABA neurons also possess ERs and may be responsible for the GnRH-1 surge. Part of the inhibitory action of endorphins on GnRH-1 release is through inhibition of these GABA neurons. LH levels are normally low during childhood and, in women, high after menopause. As LH is secreted as pulses, it is necessary to follow its concentration compared to an average cycle. The ranges denoted Inter-cycle

variability are more appropriate to use in non-monitored cycles with only the beginning of menstruation known, but where the woman accurately knows her average cycle lengths and time of ovulation, and that they are somewhat averagely regular, with the time scale being compressed or stretched to how much a woman's average cycle length is shorter or longer, respectively, than the average of the population. The ranges denoted Inter-woman variability are more appropriate to use when the average cycle lengths and time of ovulation are unknown, but only the beginning of menstruation is given. over a sufficient period of time to get proper information about its blood level. During the reproductive years, typical levels are between 1–20 IU/L. Physiologic high LH levels are seen during the LH surge (typically they last 48 hours. In males over 18 years of age, reference ranges have been estimated to be 1.8– 8.6 IU/L.LH is measured in international units (IU). In children with precocious puberty of pituitary or central origin, LH and FSH levels may be in the reproductive range instead of the low levels typical for their age. During the reproductive years, relatively elevated LH is frequently seen in patients with polycystic ovary syndrome; however, it would be unusual for them to have LH. levels outside of the normal reproductive range. Persistently high LH levels are indicative of situations where the normal restricting feedback from the gonad is absent, leading to a pituitary production of both LH and FSH. While this is typical in menopause, it is abnormal in the reproductive years. Diminished secretion of LH can result in failure of gonadal function (hypogonadism). This condition is typically manifest in males as failure in production of normal numbers of sperm. In females, amenorrhea is commonly observe (Pitteloud., 2008).

III. Prolactin (PRL) is a protein that is best known for its role in enabling mammals, usually females, to produce milk. Prolactin is secreted from the pituitary gland in response to eating, mating, estrogen treatment, ovulation and nursing. Prolactin is secreted in pulses in between these events. Prolactin plays an essential role in metabolism, regulation of the immune system and pancreatic development, stimulates the mammary glands to produce milk (lactation): increased serum concentrations of prolactin during pregnancy cause enlargement of the mammary glands and prepare for milk production, which normally starts when levels of progesterone fall by the end of pregnancy and a suckling stimulus is present. Prolactin plays an important role in maternal behavior. The hormone counteracts the effect of dopamine. Elevated levels of prolactin decrease the levels of sex hormones — estrogen in women and testosterone in men. The effects of mildly elevated levels of prolactin are much more variable, in women, substantially increasing or decreasing estrogen levels. Prolactin is sometimes classified as a gonadotropin (Hoehn and Marieb., 2007)

IV. Anti-Müllerian hormone (AMH) also known as Müllerian-inhibiting hormone (MIH), is a glycoprotein hormone structurally related to inhibin and activin from the transforming growth factor beta superfamily, whose key roles are in growth differentiation and folliculogenesis. (Rzeszowska *et al.*, 2016). AMH prevents the development of the Müllerian ducts into the uterus and other Müllerian structures. The effect is ipsilateral, that is each testis suppresses Müllerian development only on its own side. In humans, this action takes place during the first 8 weeks of gestation. If no function hormone is produced from the gonads, the Müllerian ducts automatically develop, while the Wolffian ducts, which are responsible for male reproductive parts, automatically die (Dewailly *et al.*, 2014).

V. Testosterone Testosterone daily production rate in male is 5–15mg; 2–4% of total testosterone circulates as free biologically active hormone. The rest is bound to proteins, particularly albumin and sex hormone-binding globulin (SHBG). Several equations are used to estimate free testosterone: free androgen index ((total testosterone/SHBG) × 100) is of limited value, Androstenedione is only about 6% SHBG-bound (Wass *et al*., 2014).

- **Distribution of Testosterone**

In circulation, 97.0 to 99.5% of testosterone is bound to plasma proteins, with 0.5 to 3.0% unbound. It is tightly bound to SHBG and weakly to albumin (Melmed and Conn., 2015).

- **Testosterone Transport and Metabolism**

Testosterone is converted in target tissues to the more potent androgen DHT in the presence of the enzyme 5A-reductase. There are multiple 5A-reductase isoenzymes; type 2 is the isoenzyme responsible for DHT synthesis in the genitalia, genital skin, and hair follicles. It is, therefore, essential for normal male virilization and sexual development. Testosterone may alternatively be converted into oestradiol through the action of the aromatase enzyme, found in greatest quantities in testes and adipose tissue. Many effects previously attributed to testosterone are now known to be mediated by oestrogen especially closure of epiphyses and maintenance of bone density. Testosterone and its metabolites are inactivated in the liver and excreted in the urine (Wass *et al*., 2014).

In males, 95% of circulating testosterone is derived from testicular production (3–10 mg/d). Direct secretion of testosterone by the adrenal and the peripheral conversion of androstenedione to testosterone collectively

account for another 0.5 mg/d of testosterone. Only a small amount of DHT (70 µg/d) is secreted directly by the testis; most circulating DHT is derived from peripheral conversion of testosterone. Most of the daily production of estradiol (approximately 45 µg/d) in men is derived from aromatase-mediated peripheral conversion of testosterone and androstenedione. Circulating testosterone is bound to two plasma proteins: sex hormone–binding globulin (SHBG) and albumin. SHBG binds testosterone with much greater affinity than albumin. Only 0.5–3% of testosterone is unbound. According to the "free hormone"hypothesis, only the unbound fraction is biologically active; however, albumin-bound hormone dissociates readily in the capillaries and may be bioavailable. The finding that SHBG-bound testosterone may be internalized through endocytic pits by binding to a protein called megalin have challenged the "free hormone" hypothesis. SHBG concentrations are decreased by androgens, obesity, insulin, and nephrotic syndrome. Conversely, estrogen administration, hyperthyroidism, many chronic inflammatory illnesses, and aging are associated with high SHBG concentrations. Testosterone is metabolized predominantly in the liver, although some degradation occurs in peripheral tissues, particularly the prostate and the skin. In the liver, testosterone is converted by a series of enzymatic steps that involve 5α- and 5β-reductases,3α- and 3β-hydroxysteroid dehydrogenases, and 17β-hydroxysteroid dehydrogenase into androsterone, etiocholanolone, DHT, and 3αandrostanediol. These compounds undergo glucuronida (Jameson., 2010).

- **Androgen Action**

Both testosterone and DHT exert their activity by binding to androgen receptors, the latter more avidly than testosterone. Male sexual differentiation during embryogenesis, development and maintenance of male

sex characteristics after puberty, normal male sexual function and behavior, spermatogenesis and regulation of gonadotrophin secretion (Wass *et al.*, 2014).

- **Deficiency of Testosterone**

Testosterone deficiency (also termed hypotestosteronism or hypotestosteronemia) is an abnormally low testosterone production. It may occur because of testicular dysfunction (primary hypogonadism) or hypothalamic–pituitary dysfunction (secondary hypogonadism) and may be congenital or acquired (Gould and Petty, 2000).Testosterone levels may decline gradually with age (Liverman and Blazer., 2004)

- **Medical Uses**

The primary use of testosterone is the treatment of males with too little or no natural testosterone production, also termed hypogonadism or Medical useshypoandrogenism (androgen deficiency) (Winn and Margo., 2016).

- **Androgen Production in Women**

In women, testosterone is secreted primarily by the ovaries and adrenal glands, although a significant amount is produced by the peripheral conversion of androstenedione and DHEA. Ovarian androgen production is regulated by luteinizing hormone, whereas adrenal production is ACTH-dependent. The predominant androgens produced by the ovaries are testosterone and androstenedione, and the adrenal glands are the main source of DHEA. Circulating testosterone is mainly bound to sex hormone-binding globulin (SHBG), and it is the free testosterone which is biologically active. Testosterone is converted to dihydrotestosterone in the skin by the enzyme 5A-reductase. Androstenedione and DHEA are not significantly protein-bound (Wass *et al*., 2014).

Testosterone supplementation in low doses is effective in the short-term for hypoactive sexual desire disorder in women. However, its long-term safety is unclear. Treating low androgen levels with testosterone is not generally recommended in women when it is due to hypopituitarism, adrenal insufficiency, or following surgical removal of the ovaries. It is also not usually recommended for improving cognition, the risk of heart disease, bone strength, or for generalized well being (Wierman *et al.*, 2014).

2.2.3 Obese Women and Infertility

Numerous studies report that women who are overweight or obese tend to have a more difficult time becoming pregnant than normal weight women. Moreover, once pregnancy occurs, obese women have a higher rate of pregnancy loss. Being overweight can also lead to abnormal hormone issues affecting reproductive processes for both women and men. Abnormal hormone signals, as a result of excess weight, negatively impact ovulation and sperm production. In women, it can cause the overproduction of insulin, which may cause irregular ovulation. There is also a link between obesity, excess insulin production and the infertility condition known as polycystic ovarian syndrome (PCOS). PCOS is a specific medical condition associated with irregular menstrual cycles, anovulation (decreased or stopped ovulation), obesity and elevated levels of male hormones (Karen *et al.*, 2007).

2.2.4. Obese Men and Infertility

National Institute of Environmental Health Sciences (NIEHS) are confirming that men with increased body mass indexes (BMI) are significantly more likely to be infertile than normal-weight men. The NIEHS data suggests that a 20-pound increase in a man’s weight may increase the chance of infertility by about 10 percent (Karen *et al.*, 2007).

Hormone irregularities in men affect stimulation of the testicles that inhibit sperm production. Excess fat actually causes the male hormone, testosterone, to be converted into estrogen, and those estrogens decrease testicle stimulation. Researchers from Reproductive Biology Associates report that a high BMI in men correlates with reduced testosterone levels. The study showed overweight men to have testosterone levels 24 percent lower than men of normal weight, and obese men to have levels 26 percent lower. Men with high BMIs typically are found to have an abnormal semen analysis as well (Karen *et al.*, 2007).

In men with mild to moderate obesity, SHBG levels decrease in proportion to the degree of obesity, resulting in lower total testosterone levels. However, free testosterone levels usually remain within the normal range. The decrease in SHBG levels is caused by increased circulating insulin, which inhibits SHBG production. Estradiol levels are higher in obese men compared to healthy, non obese controls, because of aromatization of testosterone to estradiol in adipose tissue. Weight loss is associated with reversal of these abnormalities including an increase in total and free testosterone levels and a decrease in estradiol levels. A subset of massively obese men may have a defect in the hypothalamic-pituitary axis as suggested by low free testosterone in the absence of elevated gonadotropins. Weight gain in adult men can accelerate the rate of age-related decline in testosterone levels (Jameson. 2010).

2.2.5 Testosterone Assay

- **Measurement of Total Testosterone**

Total testosterone includes both unbound and protein bound testosterone and is measured by radioimmunoassays, immunometric assays, or liquid chromatography tandem mass spectrometry (LC-MS/MS). LC-MS/MS

involves extraction of serum by organic solvents, separation of testosterone from other steroids by high-performance liquid chromatography and mass spectrometry, and quantitation of unique testosterone fragments by mass spectrometry. LCMS/MS provides accurate and sensitive measurements of testosterone levels even in the low range and is emerging as the method of choice for testosterone measurement. A single random sample provides a good approximation of the average testosterone concentration with the realization that testosterone levels fluctuate in response to pulsatile LH. Testosterone is generally lower in the late afternoon and is reduced by acute illness. The testosterone concentration in healthy young men ranges from 300 to 1000 ng/dL in most laboratories, although these reference ranges are not derived from population-based random samples. Alterations in SHBG levels due to aging, obesity, some types of medications, or chronic illness, or on a congenital basis, can affect total testosterone levels (Jameson., 2010).

- **Measurement of Unbound Testosterone Levels**

Most circulating testosterone is bound to SHBG and to albumin; only 0.5–3% of circulating testosterone is unbound, or "free."The unbound testosterone concentration can be measured by equilibrium dialysis or calculated from total testosterone, SHBG, and albumin concentrations by using published mass-action equations. Tracer analogue methods are relatively inexpensive and convenient, but they are inaccurate. Bioavailable testosterone refers to unbound testosterone plus testosterone that is loosely bound to albumin; it can be estimated by the ammonium sulfate precipitation method (Jameson., 2010).

Chapter Three

Materials and Methods

3. Materials and Methods

3.1 Study Approach

Quantitative methods were used to estimate testosterone hormone in obese subjects in Khartoum state.

3.2 Study Design

This was cross sectional case-control study.

3.3 Study Area

This study was conducted in Khartoum State during the period from August to November 2017.

3.4 Study Populations:

The study included 100 individuals, 50(obese and overweight) male and female as cases and 50 normal body weight subjects serve as control (age and sex were matched).

3.5 Inclusion Criteria

Healthy obese Sudanese subjects and normal body weight healthy individuals (normal BMI) serve as control were included.

3.6 Exclusion Criteria

Diabetic, hypertensive patients, pregnant women and patients with endocrinopathy were excluded.

3.7 Ethical Consideration

Before the samples were collected, the donors knew about the objectives of the study and verbal informed consent was obtained.

3.8 Data Collection

The clinical data were obtained from clinical examinations and were recorded on a questionnaire sheet (Appendix1).

3.9 Samples Collection and Processing:

About 4 ml of venous blood were collected from each participant (both cases and controls). The samples collected under aseptic conditions and placed in sterile Plain containers, and after mixing centrifuged for 10 minutes at 3000 rpm to obtain Serum, then the serum were kept at $^{-}20^{o}C$ till the time of analysis.

3.10 Estimation of Testosterone:

3.10.1 Principle of the Method

A competition method with a final fluorescent detection (ELFA), in which testosterone in the serum competes with testosterone derivative in the conjugate for the anti- testosterone specific antibody sites coated to the inner surface of the SPR. The conjugate enzyme is catalyzes the hydrolysis of the substrate 4-Methyl-umbelliferyl phosphate into a fluorescent product 4-Methyl-umbelliferone, the fluorescent of which is measured at 450nm, the intensity of fluorescence is inversely proportional to the concentration of antigen present in the sample. At the end of the assay the result are automatically calculated by the instrument in relation to the calibration curve stored in memory.

Procedure:

The sample was transferred into the well containing the conjugate, which is an alkaline phosphatase - labeled testosterone derivative. Testosterone is present in the serum competes for the anti- testosterone specific antibody sites coated to the inner surface of the SPR. at the end of the assay the result are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

For reagent preparation see appendix II.

3.3 Quality Control

The precision and accuracy of all methods used in this study were checked by commercially prepared control sample (control normal and pathogen) before its application for the measurement of test and control samples.

3.4 Data Analysis

Data was analyzed to obtain means standard deviation and correlation of the sampling using statistical package for social science (SPSS) computer Programmed version 11.5, t test and Person correlation were applied for correlation between variables.

Chapter Four

Results

4. Results

The results of testosterone hormone estimation in obese, overweight and control are given in tables and Figures as follow:

(Figure 4-1): shows the distribution of the study group according to gender, male (40%) and female (60%).

(Table 4-1): shows that there is significant difference between the means of BMI in obese (32.9 ± 1.9) ng/dl, overwight (27.3 ± 1.3)) ng/dl, and control (22.7 ± 1.3)) ng/dl, with *P.value* (0.00).

(Table 4-2):shows that there is significant difference between means of class1 obese in compare to control with *P.value* (0.00), and a signifinat difference between class2 obese in compare to control with *P.value(*0.00).

(Table 4-3): shows that mean concentration of testosterone in obese (4.25±1.97)) ng/dl, was significantly decreased compared to control (9.97±10.74)) ng/dl, with *P.value* (0.003), the mean concentration of testosterone in overweight (8.57±5.37)) ng/dl, was insignificantly decreased compared to control (9.97±10.74)) ng/dl, with *P.value* (0.513), and the mean concentration of testosterone in obese (4.25±1.97)) ng/dl, was insignificantly decreased compared to overweight (8.57±5.37)) ng/dl, with with *P.value (*0.067).

(Table 4-4): Shows that mean concentration of testosterone in obese male (4.17±1.05)) ng/dl, was significantly decreased compared to control male (22.05±6.34)) ng/dl, with *P.value* (0.00), the mean concentration of testosterone in overweight male (12.50 ±4.81)) ng/dl, was significantly decreased compared to control (22.05±6.34) with *P.value* (0.00), and the mean concentration of testosterone in obese male (4.17±1.05)) ng/dl, was significantly decreased compared to overweight male (12.50 ±4.81)) ng/dl, with *P.value* (0.001).

(Table 4-5): Shows that mean concentration of testosterone in obese female (4.70±2.97)) ng/dl, was significantly increased compared to control (1.92 ± 0.89) with *P.value* (0.00), the mean concentration of testosterone in overweight female (3.70 ±1.45)) ng/dl, was significantly increased compared to control (1.92 ± 0.89)) ng/dl, with *P.value* (0.012), and the mean concentration of testosterone in obese female (4.70±2.97)) ng/dl, was insignificantly increased compared to overweight female (3.70 ±1.45) ng/dl, with with *P.value* (0.183).

(Table 4-6): Shows that mean concentration of testosterone in obese class 1(3.95±1.89)) ng/dl, was significantly decreased compared to control (9.97 ± 10.74)) ng/dl, with *P.value* (0.008), the mean concentration of testosterone in obese class 2 (5.10 ±4.58)) ng/dl, was insignificantly decreased compared to control (9.97 ± 10.74)) ng/dl, with *P.value* (0.170), and the mean concentration of testosterone in obese class1 (3.95±1.89) ng/dl, was insignificantly decreased compared to obese class 2 (5.10 ±4.58) ng/dl, with with*P.value* (0.760).

(Figure 4-2): shows strong positive correlation between BMI and Testosterone hormone in obese female(r=0.5, p=0.03).

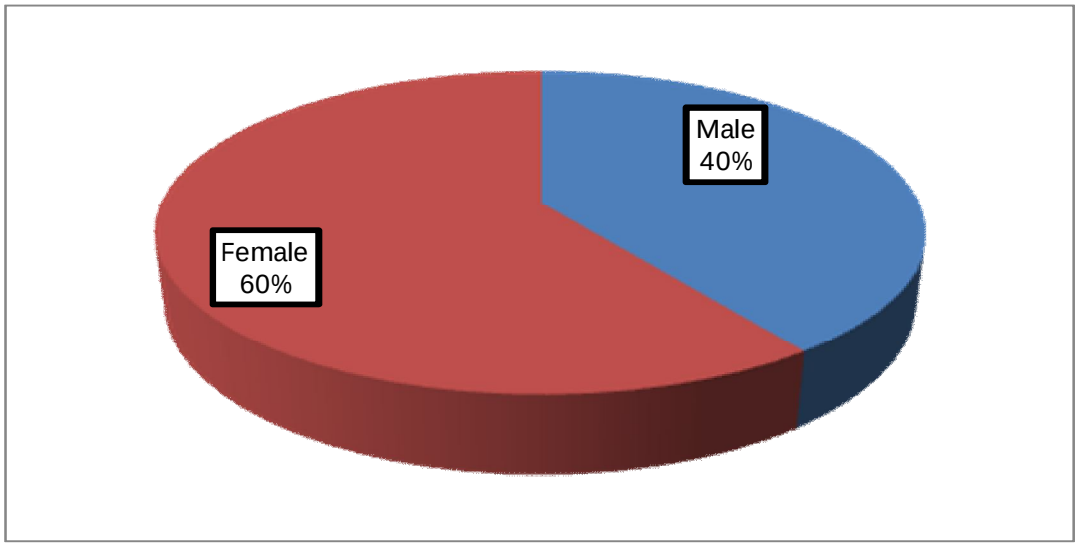

Figure (4-1) Study group distribution according to gender

(Table 4-1): Comparison between Means of BMI among the control group and obese and overweight:

BMI	**Mean ± Sd (ng/dl)**	***p.value***
Obese (n=30) Control (n=50)	32.94 ± 1.93 22.72 ± 1.32	0.00*
Overweight (n=20) Control (n=50)	27.34 ± 1.32 22.72 ± 1.32	0.00*
Obese (n=30) Overweight (n=20)	32.94 ± 1.93 27.34 ± 1.32	0.00*

-Independent sample T test was used.

- *p.value* considered significant at level ≥ 0.05.

Table (4-2): Comparison between BMI Means in the Study Group with the Control Group According to the class of Obesity:

Obese Classification	**Mean ± Sd (ng/dl)**	***P. value***
Class 1 (n= 23) Control (n=50)	32.12 ± 1.30 22.7 ± 1.32	0.00*
Class 2 (n=7) Control (n=50)	35.63 ± 0.84 22.72 ± 1.32	0.00*

- Independent sample T test was used.
- *p.value* considered significant at level ≥ 0.05.

Table (4-3): Comparison between Means of Serum Testosterone Level Among Obese Sudanese Male to Control male Group:

Male Testosterone	**Mean ± Sd (nmol/l)**	***P-value***
Obese (n=10) Control (n=20)	4.17±1.05 22.05±6.34	0.00
Overweight (n=10) Control (n=20)	12.50 ±4.81 22.05±6.34	0.00
Obese (n=10) Overweight (n=10)	4.17±1.05 12.50 ±4.81	0.001

-ONE WAY ANOVA T test was used.

-*p.value* considered significant at level ≥ 0.05.

Table (4-4): Comparison between Means of Serum Testosterone level Among Obese Female Sudanese to Control Group:

Female Testosterone	**Mean ± Sd (nmol/l)**	***P-value***
Obese (n=20) Control (n=30)	4.70 ±2.97 1.92 ± 0.89	0.00*
Overweight (n=10) Control (n=30)	3.70 ±1.45 1.92 ± 0.89	0.012*
Obese (n=20) Overweight (n=10)	4.70 ±2.97 3.70 ±1.45	0.183

-ONE WAY ANOVA T test was used.

-*p.value* considered significant at level ≥ 0.05.

Table (4-5): Comparison between Means of Testosterone Hormones among Obese Classes I and II with that of Control Group:

Testosterone	**Mean ± Sd (nmol/l)**	***P-value***
Obese class 1(n=23) Control (n=50)	3.95 ±1.98 9.97 ± 10.74	0.008*
Obese class 2 (n=7) Control (n=50)	5.10 ±1.89 9.97 ± 10.74	0.170
Obese class 1(n=23) Obese class 2(n=7)	3.95 ±1.98 5.10 ±1.89	0.760

-ONE WAY ANOVA T test was used.

-*p.value* considered significant at level ≥ 0.05.

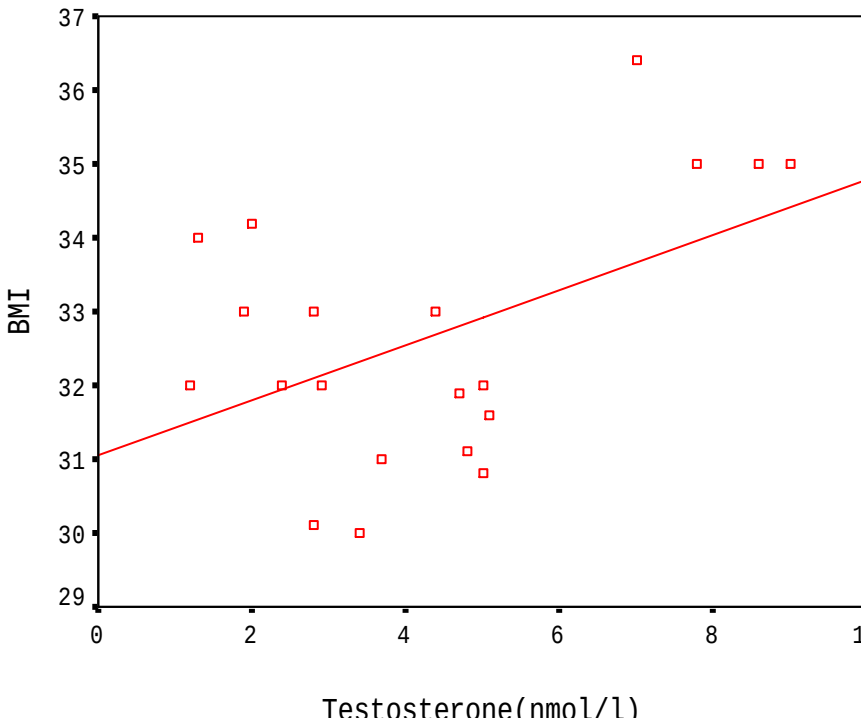

Figure (4-2): Scatter plot between serum Teststosterone and BMI among Obese female. (r=0.5, p=0.03)

Chapter Five

Discussion, Conclusion and Recommendations

5. Discussion, Conclusion and Recommendations

5.1 Discussion

Body fat distribution plays an important role in the development of obesity-related conditions. Fat around our abdomen is a higher risk factor for disease than fat stored on our bottom, hips and thighs. It seems that oestrogens and androgens help to decide body fat distribution (lovejoy and Sainsbury, 2008)

Disorders that affect the reproductive axis are associated with obesity in both men and women. Male hypogonadism is associated with increased adipose tissue, Obesity has long been associated with menstrual abnormalities in women, a common finding is increased androgen production (Jameson. 2010).

Obesity affects testosterone hormone in the body, this study conducted to get the effect of obesity on the level of serum testosterone.

The finding obtained from especially designed questionnaire revealed that, testosterone hormones decreased in obese male, this finding agreed with study carried by (Pasquali, 2003), which showed that total testosterone is significantly decreased in obese male in proportion to degree of obesity. Testosterone is increased in obese female (BMI $\geq$ 30) which agreed with study carried by (Pasquali, 2003; Kirschner *et al,* 1990), who showed that obese women tend to develop a condition of hyperandrogenism.

Also testosterone hormone significantly decreases in overweight male, and significantly increases in overweight female in compare to control group.

From the findings of this study, it appears that serum testosterone level significantly decreases in obese male and significantly increases in obese female with p.vale (0.00), this agreed with study by (Pasquali, 2003) who showed that reduced sex hormone–binding globulin synthesis and

circulating blood levels represent the sole common mechanism which is responsible in both sexes.

Among other still partially undefined factors, mechanisms potentially responsible for the sex dichotomy in androgen levels involve specific alterations of gonadotropin secretion, estrogens, the hypothalamic-pituitary-adrenal axis, leptin, androgen receptors, specific steroidogenic enzymes in the peripheral tissues, and, possibly, ghrelin. In both sexes, androgens play an important role in determining the sex-dependent pattern of body fat distribution, also agreed with study by (Arjonilla and Schwarcz, 2009), who showed that low serum SHBG levels in obesity contribute to the low serum total testosterone in men.

5.2 Conclusion

From the results and finding of this study, it is concluded that:

The level of serum testosterone is significantly decreased in obese male, and significantly increased in obese female, it is insignificantly decreased in overweight male, and insignificantly increased in overweight female.

5.3 Recommendations

From the findings of this study it is recommended that:

1) Awareness about obesity, its complications and effects on fertility should be started.
2) The level of serum testosterone should be checked in obese infertile male and female.
3) Treatment of infertility should be accompanied by diet under medical control.

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