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 ovariansyndrome (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 estradiol hormone level and obesity.

And there are no published studies about this in Sudan, it may help to provid rephrase and monitoring of estradiol hormones in obese subjects.

1.3 Objectives:

1.3.1 General objective

❖ To evaluate estradiol hormone level in healthy obese subjects in Khartoum state.

1.3.2 Specific objectives

1- To estimate and compare estradiol hormone level in study groups.

2- To correlate estradiole hormone level with BMI and in obese classes

3- To compare between estradiol hormone and sex.

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 adult women 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 of obesity

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 of obesity

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 of obesity:

❖ 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 acanthosis nigricans, 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, acanthosis nigricans, 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

(skinfold 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 on this 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 of obesity**

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 of obesity**

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

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 longterm. 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. Estrogen

Estrogen, is the primary female sex hormone as well as a medication. It is responsible for the development and regulation of the female reproductive system and secondary sex characteristics. The estradiol is the most potent and prevalent endogenous estrogen, although a number of other endogenous compounds also have estrogenic hormonal activity (Mechoulam., 2005).

- **Production of Estrogen**

Oestradiol daily production rate in male is 40–400 micrograms; 2–3% free oestradiol is biologically active; the rest is bound to SHBG (John *et al*., 2014).

- **Types of Estrogen**

The three major naturally occurring estrogens in women are estrone (E1),estradiol (E2), and estriol (E3). Estradiol is the predominant estrogen during reproductive years both in terms of absolute serum levels as well as in terms of estrogenic activity. During menopause, estrone is the predominant circulating estrogen and during pregnancy estriol is the predominant circulating estrogen in terms of serum levels. Though estriol is the most plentiful of the three estrogens it is also the weakest, whereas estradiol is the strongest with a potency of approximately 80 times that of estriol (Files., 2011).

Thus, estradiol is the most important estrogen in non-pregnant females who are between the menarche and menopause stages of life. However, during pregnancy this role shifts to estriol, and in postmenopausal women estrone becomes the primary form of estrogen in the body. Another type of estrogen called estetrol (E4) isproduced only during pregnancy.

- **Synthesis of Estrogen**

Estrogens are synthesized in all vertebrates as well as some insects.Their presence in both vertebrates and insects suggests that estrogenic sex hormones have an ancient evolutionary history. The three major naturally occurring forms of estrogen in women are estrone (E1), estradiol (E2), and estriol (E3). Another type of estrogen called estetrol (E4) is produced only during pregnancy. While estrogen levels are significantly lower in males compared to females, estrogens nevertheless also have important physiological roles in males (Lombardi., 2001).

All of the different forms of estrogen are synthesized from androgens, specifically testosterone and androstenedione, by the enzyme aromatase. Minor endogenous estrogens, the biosyntheses of which do not involve aromatase, include 27-hydroxycholesterol, dehydroepiandrosterone (DHEA), 7-oxoDHEA, 7α-hydroxy-DHEA, 16α-hydroxy DHEA, 7β-hydroxyepiandrosterone, androstenedione (A4), androstenediol (A5), 3α-androstanediol, and 3βandrostanediol.Some estrogen metabolites, such as the catechol estrogens 2-hydroxyestradiol, 2hydroxyestrone, 4-hydroxyestradiol, and 4hydroxyestrone, as well as 16αhydroxyestrone, are also estrogens with varying degrees of activity.The biological importance of these minor estrogens is not entirely clear (Bahavanani., 2000).

- **Metabolism of Estrogen**

Oestradiol is conjugated in the liver to sulphates and glucuronates and then extracted in the urine (John *et al*., 2014).

- **Estrogen Action**

Estradiol binds to A (reproductive tissues) and B (bone, brain, heart, etc.) receptors, it develops of male sex characteristics, it increases fat stores and Increases vaginal wall and uterine thickening (John *et al*., 2014).

The actions of estrogen are mediated by the estrogen receptor (ER), a dimeric nuclear protein that binds to DNA and controls gene expression. Like other steroid hormones, estrogen enters passively into the cell where it binds to and activates the estrogen receptor. The estrogen:ER complex binds to specific DNA sequences called a hormone response element to activate the transcription of target genes(Lin., 2004).

It mediates formation of female secondary sex characteristics. Accelerates metabolism, Increases fat store, Stimulates endometrial growth, Increases uterine growth, Increases vaginal lubrication, Thicken the vaginal wall, maintenance of vessel and skin, Reduces bone resorption, increase bone formation Protein synthesis, Increases hepatic production of binding proteins coagulation, Increases circulating level of factors 2, 7, 9, 10, plasminogen, decreases antithrombin III, increases platelet adhesiveness, increases HDL, triglyceride, decreases LDL, fat deposition, Salt (sodium) and water retention, Increase cortisol, Reduces bowel motility, Increases cholesterol in bile, increases pheomelanin, reduce eumelanin, supports hormone-sensitive breast cancers Promotes lung function by supporting alveoli (in rodents but probably in humans (Massaro., 2004)

2.2.3 Obesity and Estrogen

Obesity can also be considered a condition of exaggerated estrogen production. It has been demonstrated that the conversion of androgens to estrogen in peripheral tissues is significantly correlated with body weight and the amount of body fat .Several other factors can contribute to this condition of 'functional hyperestrogenism ((Pasquali and Casimirri., 1993). Due to reduced SHBG synthesis and lower circulating SHBG concentrations in obesity, the free estradiol fraction increases, thus increasing exposure of target tissues to this hormone. Moreover, the metabolism of estrogens is altered in obese women. A decreased formation of inactive estradiol metabolites, such as 2-hydroxyestrogens, which are virtually devoid of peripheral estrogen activity, is observed, together with a higher than normal production of estrone sulfate (which represents an important reservoir of active estrogens, particularly estrone), due to the concurrent reduction of its metabolic clearance and increased production rate. The final result of these metabolic derangements on estrogens is an increased ratio of active to inactive estrogens in obese women (Kurtz., 1987).

3. Materials and Methods

3.1 Study Approach

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

3.2 Study Design

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 included100 individuals, 50(obese and overweight) male and female as cases and 50 normal body weight subjects serve as control (age and sex were matched).their average age between (18 to 45) years.

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 Considerations

Before the samples were collected, the donors informed 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 (Appendix I).

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 $^-20^oC$ till the time of analysis.

3.10 Estimation of Estrogen:

3.10.1 Principle of the Method

A competition method with a final fluorescent detection (ELFA).The estradiol in serum compete with estradiol derivative in the conjugate for the anti- estradiol specific antibody sites coated to the inner surface of the SPR., the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme is catalyzes the hydrolysis of this substrate into a fluorescent product ((4-Methyl-umbelliferone), 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.(Appendix)

3.10.2 Procedure

The sample was transferred into the well containing the conjugate, which is an alkaline phosphatase- labeled estradiol derivative. The estradiol in serum as compete for the anti- estradiol specific antibody sites coated to the inner surface of the SPR.

The results 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.

3.11 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.

3.12 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.

4. Results

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

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

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

(**Table 4-3**): shows that the mean concentration of estradiol in obese (146.3±108.6) was significantly increased compared to control (63.4±52.8) with *P.value* (0.00), and the mean concentration of estradiol in overweight (112.5±102.3) was significantly increased compared to control (63.4±52.8) with *P.value*(0.029).

(**Table 4-4**): Shows that mean concentration of estradiol in obese male (68.20 ±39.21) was significantly increased compared to control male (36.2±13.2) with *P.value* (0.01), and the mean concentration of estradiol in overweight male (58.2 ±19.2) was significantly increased compared to control (36.2±13.2) with *P.value* (0.021) and the mean concentration of estradiol in obese male (68.2 ±39.2) was insignificantly increased compared to overweight male (58.2 ±19.2) with *P.value* (0.348).

(**Table 4-5**): Shows that mean concentration of estradiol in obese female (185.2±111.7) was significantly increased in compare to control (81.6 ±61.2) with *P.value* (0.00), and the mean concentration of estradiol in overweight female (166.7±123.2) was significantly increased in compare to control (81.6 ±61.7) with *P.value* (0.236), and the mean concentration of estradiol in obese female (185.2±111.7) was insignificantly increased in compare to overweight female (166.7±123.2) with with *P.value* (0.605).

(**Table 4-6**): Shows that mean concentration of estradiol in obese class 1(129.5±116.6) was significantly increased in compare to control(61.7±53.6) with *p. value* (0.001), and the mean concentration of estradiol in obese class 2 (182.4±87.8) was significantly increased in compare to control(61.7±53.6) with *p. value* (0.00), and the mean concentration of estradiol in obese class

2(182.4±87.8) was insignificantly increased in compare to obese class 1(129.5±116.6) with *p. value* (0.129).

(Figure 4-1): shows the distribution of male (40%) and female (60%).

Figure (4-2): shows strong positive correlation between BMI and Estradiol in obese male(r=0.7, p=0.03)

(Table 4-1): Comparison between Means of BMI among Study Groups:

BMI K/M²	Mean ± SD	p.value
Obese (n=30)	32.94 ± 1.93	0.00*
Control (n=50)	22.72 ± 1.32	
Overweight (n=20)	27.34 ± 1.34	0.00*
Control (n=50)	22.72 ± 1.32	
Obese (n=30)	32.94 ± 1.93	0.00*
Overweight (n=20)	27.34 ± 1.34	

-Independent sample T test was used.

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

Table (4-2): Comparison between Means of BMI among Obese Classes:

Obese classification	Mean ± SD	*P. value*
Class 1 (n= 23)	32.1 ± 0.39	0.00*
Control (n=50)	22.7 ± 1.32	
Class 2 (n=7)	35.6 ± 0.84	0.00*
Control (n=50)	22.7 ± 1.32	

- Independent sample T test was used.

P.value considered significant at level ≥ 0.05.

Table(4-3): Comparison between Means of Serum Estradiol among Study Groups:

BMI K/M²	Mean ± SD(Pg/ml)	*p.value*
Obese (n=30)	146.28±108.64	
Control(n=50)	63.42±52.78	0.00*
Overweight(n=20)	112.45±102.27	
Control(n=50)	63.42±52.78	0.029*
Obese (n=30)	146.28±108.64	0.165
Overweight (n=20)	112.45±102.27	

-Independent sample T test was used.

P.value considered significant at level ≥ 0.05.

Table(4-4): Comparison between Means of Estradiol in Male Study Group:

Male estradiol	Mean ± SD (pg/ml)	*P-value*
Obese (n=10)	68.20 ±39.21	0.01*
Control (n=20)	36.20 ±13.19	
Overweight (n=10)	58.20 ±19.24	0.021*
Control (n=20)	36.20 ±13.19	
Obese (n=10)	68.20 ±39.21	0.348
Overweight (n=10)	58.20 ±19.24	

-ONE WAY ANOVA T test was used.

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

Table(4-5): Comparison between Means of Estradiol in Female Study Group:

Female estradiol	Mean ± SD (pg/ml)	*P-value*
Obese (n=20)	185.22 ± 111.73	0.00*
Control (n=30)	81.57 ±61.17	
Overweight (n=10)	166.70 ±123.18	0.236
Control (n=30)	81.57 ±61.17	
Obese (n=20)	185.22 ± 111.73	0.605
Overweight (n=10)	166.70 ±123.18	

-ONE WAY ANOVA T test was used.

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

Table(4-6): Comparison between Means of Estradiol in Obese Classes:

Estradiol	Mean ± SD (pg/ml)	*P-value*
Obese class 1 (n=23)	129.49 ± 116.58	0.001*
Control (n=50)	61.56 ±53.59	
Obese class 2 (n=7)	182.14 ±87.84	0.00*
Control (n=50)	61.56 ±53.59	
Obese class 1 (n=23)	129.49 ± 116.58	0.129
Obese class 2(n=7)	182.14 ±87.84	

-ONE WAY ANOVA T test was used.

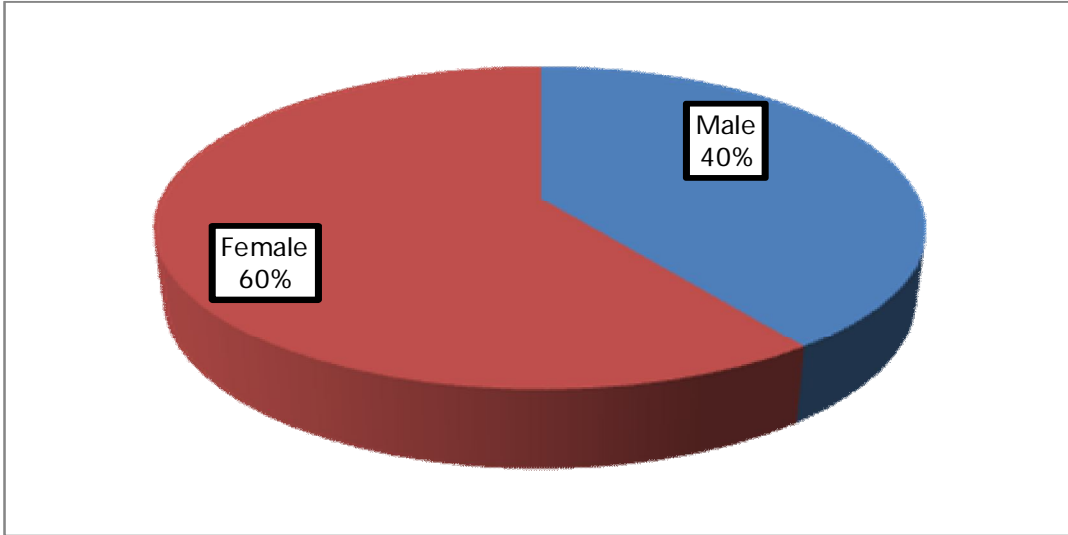-*p.value* considered significant at level ≥ 0.05.

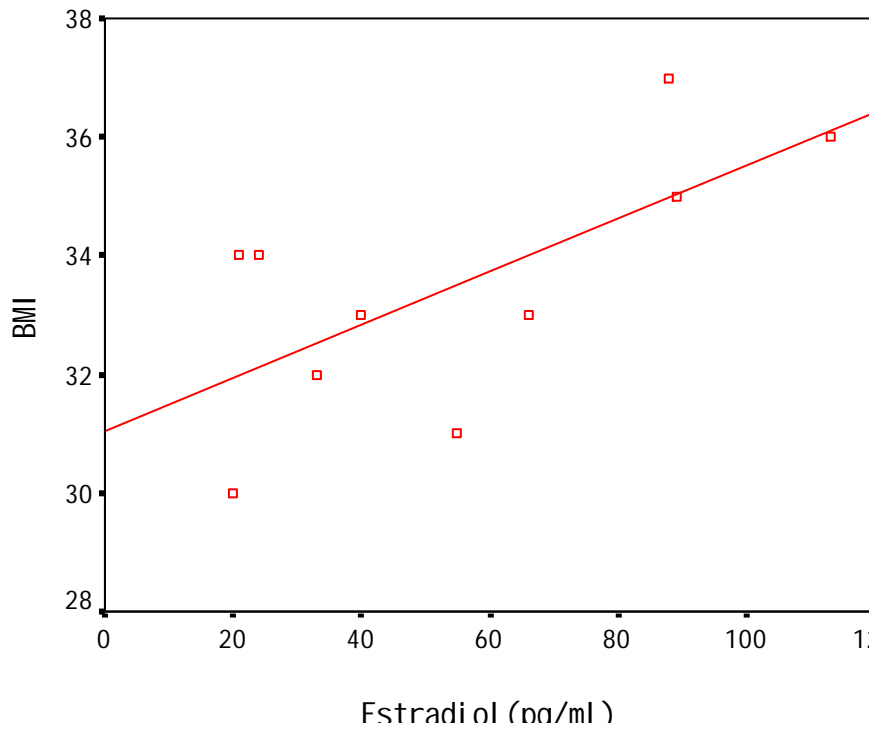**Figure (4-1) Gender distribution among study group**

Figure (4-2): shows correlation between BMI and Estradiol in obese male(r=0.7, p=0.03)

5. Discussion, Conclusion and Recommendations

5.1 Discusion

Body fat distribution plays an important role in the development of obesity-related conditions. Fat around abdomen is a higher risk factor for disease than fat stored on bottom, hips and thighs. It seems that oestrogens and androgens help to decide body fat distribution (lovejoy and Sainsbury, 2008) A study conducted to evaluate serum estradiol level in obesity demonstrated that estradiol hormone increased in obese female this finding agreed with study carried by (Kirschner *et al.,* 1990), who conducted a study on androgen-estrogen on obese women with upper or lower body obesity that shows that women with upper body obesity were observed to have higher serum estradiol (E2).

In addition it showed that estradiol increased in obese males of which agreed with study done by (Zumoff., 1982), who reported that obese young men have elevated plasma estrogen level but obese women not.

From the findings of this study, it appears that serum estradiol level significantly increases in male *with p.*value (0.01) and female with p.vale (0.00). A study by (Kim *et al,* 2014) explaines the mechanism by which estradiol affects adipose tissue the female sex hormone, estrogen, regulates adipose development and improves systemic glucose homeostasis in both males and females. The underlying mechanism linking estrogenic regulation in adipose tissue and systemic glucose metabolism has not been fully elucidated, but is thought to include interactions of estrogen receptor signaling events involving lipolytic and/or lipogenic enzyme activity, free fatty acid metabolism, and adipocytokine production.

5.2 Conclusion

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

The level of serum estrogen is increased in obese and overweight male and female.

5.3 Recommendations

From the findings of this study it is recommended that:

1. The level of serum estrogen should be checked in obese male and female.

2. Treatment of infertility should be accompanied by diet under medical control

References

- **Aled. R., Miles. L., Andrew. L;** (2017)."*Clinical endocrinology and diabetes*: at a glance". Chichester: Willey Black Well.

- **Aune. D., Sen. A., Prasad. M., Norat. T., Janszky. I., Tonstad. S., Romundstad. P., Vatten. L.J;** (2016). "BMI and all cause mortality: systematic review and non-linear dose-response meta-analysis of 230 cohort studies with 3.74 million deaths among 30.3 million participants*". BMJ* (Clinical research Ed.).

- **Barness. L.A., Opitz. J.M., Gilbert-Barness. E;** (2007). "Obesity: genetic, molecular, and environmental aspects". *American Journal of Medical Genetics.* (24): 3016–34.

- **Ben. G.,Diana. W;** (2011)."*The endocrine system*: at a glance ".3rd (Ed). Chichester: Willey Black Well.

- **Berrington de Gonzalez. A., Hartge. P., Cerhan. J.R., Flint. A.J., Hannan. L., MacInnis. R.J** *et al;* (2010). "Body-mass index and mortality among 1.46 million white adults. *The New England Journal of Medicine.* 363 (23): 2211–9.

- **Bhavnani. B.R., Nisker. J.A., Martin. J., Aletebi. F., Watson. L., Milne. J.K;** (2000). "Comparison of pharmacokinetics of a conjugated equine estrogen preparation (premarin) and a synthetic mixture of estrogens (C.E.S.) in postmenopausal women". *J. Soc. Gynecol. Investig.* 7 (3): 175–83.

- **Carmienke. S., Freitag. M.H., Pischon. T., Schlattmann. P., Fankhaenel. T., Goebel. H., Gensichen.J;** (2013). "General and abdominal obesity parameters and their combination in relation to

mortality: a systematic review and meta-regression analysis". *European Journal of Clinical Nutrition*. 67 (6): 573–585

- **Dewailly. D., Andersen. C.Y., Balen. A., Broekmans. F., Dilaver. N., Fanchin. R., Griesinger. G** *et al* **;** (2014). "The physiology and clinical utility of antiMullerian hormone in women". Human Reproduction Update. 20 (3): 370–385.

- **Flegal. K.M., Kit Brian. K., Orpana. H., Graubard. B.I;** (2013). "Association of All-Cause Mortality With Overweight and Obesity Using Standard Body Mass Index Categories". *JAMA*. 309 (1): 71–82.

- **Flier. J.S;** (2004). *"*Obesity wars: Molecular progress confronts an expanding epidemic". *Cell (Review)*. 116 (2): 337–50.

- **Green. D.J;** (2009). Is body mass index really the best measure of obesity in individuals? *J Am Coll Cardiol.*10;53(6):526.

- **Haslam. D.W., James. WP;** (2005). "Obesity". *Lancet (Review)*. 366 (9492): 1197–209.

- **Hoehn. K., Marieb. E.N;** (2007). Human Anatomy & Physiology. San Francisco: Pearson Benjamin Cummings. p. 605

- **John. W., Katharine. O;** (2014)." Oxford handbook of endocrinology and diabetes" (eds). 3rd Ed.US: Oxford.

- **Kanazawa. M., Yoshiike. N., Osaka. T., Numba. Y., Zimmet. P., Inoue. S;** (2005). "Criteria and classification of obesity in Japan and Asia-Oceania*". World review of nutrition and dietetics*. 94: 1–12.

- **Kim. J.H., Cho. H.T., Kim. Y.J;** (2014). The role of estrogen in adipose tissue metabolism: insights into glucose hemostasis regulation**.** *J-stage***.11**:1055-1067

- **Kirschner. M.A., Samojlik. M., Drejka. E., Szmal.G., Schneiedr. N;** (1990). Androgen-estrogen metabolism in women with upper body versus lower body obesity. *The journal of clinical endocrinology and metabolism.***70**(2):473-479.

- **Lin. C.Y., Ström, A., Vega. V.B., Kong. S.L., Yeo. A.L., Thomsen. J.S., Chan.W.C., Doray. B., Bangarusamy. D.K., Ramasamy. A.,Vergara. L.A., Tang. S., Chong. A., Bajic. V.B., Miller. L.D., Gustafsson. J.A., Liu. E.T;** (2004). "Discovery of estrogen receptor alpha target genes and response elements in breast tumor cells". *Genome Biol.* 5 (9): R66.

- **Lombardi. G., Zarrilli. S., Colao. A., Paesano. L., Di Somma. C., Rossi. F., De Rosa. M;** (2001*). "*Estrogens and health in males". *Molecular and Cellular Endocrinology*. 178 (1–2): 51.

- **Lovejoy. J.C., Sainsbury, A;** (2008) 'Sex differences in obesity and the regulation of energy homeostasis', *Obesity Review*, **10**(2): 154–167.

- **Mahadevan.H. E; Vishy;** (2013). *Clinical anatomy applied anatomy for students and junior doctors* (13th ed.). Chichester, West Sussex, UK: WileyBlackwell

- **Mahesh .V.B;** (January 2012). "Hirsutism, virilism, polycystic ovarian disease, and the steroid-gonadotropin-feedback system: a career retrospective" . American Journal of Physiology. Endocrinology and Metabolism. 302 (1): E4–E18.

- **Marieb. E;** (2013). Anatomy & physiology. *Benjamin-Cummings*. p. 915.

- **Massaro. D., Massaro. G.D;** (2004*).* "Estrogen regulates pulmonary alveolar formation, loss, and regeneration in mice". *Am. J. Physiol. Lung Cell Mol. Physiol*. 287 (6): L1154–9.

- **Mayes.H.A., Watson.G.H;** (2004).Direct effects of sex streroids hormones on adipose tissue and obesity.*obesity review* **5**(4).

- **Mechoulam. R., Brueggemeier. R.W., Denlinger. D.L;** (2005). "Estrogens in insects". Cellular and Molecular Life Sciences. 40 (9): 942–944

- **Mørkedal. B; Romundstad. P.R; Vatten. L.J;** (2011). "*Informativeness of indices of blood pressure, obesity and serum lipids in relation to ischaemic heart disease mortality*: the HUNT-II study". European Journal of Epidemiology. 26 (6): 457–461.

- **NCBI**; (2016).CGA glycoprotein hormones, alpha polypeptide *[Homo sapiens (human)]".*

- **Peter. G;** (2000*).*"*Obesity as a medical proplem*". *Nature*.404(6)635-643

- **Pischon. T., Boeing. H., Hoffmann. K., Bergmann. M., Schulze. M.B., Overvad. K., van der Schouw. Y.T., Spencer. E., Moons. K.G., Tjønneland. A.,** (2008). "*General and abdominal adiposity and risk of death in Europe*".359 (20): 2105–20.

- **Pitteloud. N., Dwyer. A.A., DeCruz. S., Lee. H., Boepple. P.A., Crowley. W.F., Hayes. F.J;** (2008). "Inhibition of luteinizing hormone secretion by testosterone in men requires aromatization for its pituitary but not its hypothalamic effects: evidence from the tandem study of normal and gonadotropin-releasing hormone-decient men" . The Journal of Clinical Endocrinology and Metabolism. 93 (3): 784–91.

- **Price. G.M., Uauy. R., Breeze. E., Bulpitt. C.J., Fletcher. A.E;** (2006). "Weight, shape, and mortality risk in older persons: elevated waist-hip ratio, not high body mass index, is associated with a greater risk of death". *Am. J. Clin. Nutr*. 84 (2): 449–60.

- **Radu. A., Pichon. C., Camparo. P., Antoine. M., Allory. Y., Couvelard. A., Fromont. G., Hai. M.T., Ghinea. N;** (2010). "Expression of follicle-stimulating hormone receptor in tumor blood vessels". N. Engl. J. Med. 363 (17): 1621–30.

- **Renato. P., Valentina. V;** (2001)." Obesity and hormonal abnormalities". *International text book of obesity*.[ed].p.225-239

- **Rzeszowska.M., Leszcz. A., Putowski. L., Hałabiś. M., Tkaczuk-Włach. J., Kotarski. J., Polak. G;** (2016). "Anti-Müllerian hormone: structure, properties and appliance" . Ginekologia Polska. 87 (9): 669–674.

- **Sharma. T. P.,Nett.T. M., Karsch. F. J., Phillips. D. J .,Lee. J. S. Herkimer. C .V;** (2012) [Published online before print 2012-03-14]. "Neuroendocrine Control of FSH Secretion: IV. Hypothalamic Control of Pituitary FSHRegulatory Proteins and Their Relationship to Changes in FSH Synthesis and Secretion" . Biology of Reproduction. the Society for the Study of Reproduction . 86 (6). article 171, p. 1-9.

- **Singh;** (1993). "Body shape and women's attractiveness. The critical role of waist-to-hip ratio". *Human Nature*. 4 (3): 297–321

- **Thomas. F., Antonia. B., Bijay. V;** (2015*). "Eureka endocrinology*". London: JP Medical.

- **Tworoger. S.S., Missmer. S.A., Barbieri. R.L., Willet. W.C., Colditz. G.A., Hankinson. S.E;** (2005). Plasma sex hormone concentrationsand

subsequent risk of breast cancer among women using postmenopausal hormones. *Journal of the national cancer Institute.* (8):595-602

- **World Health Organization (WHO);** (2008)."*Waist Circumference and Waist-Hip Ratio*, Report of a WHO Expert Consultation".
- **Zumoff. B;** (1982). Relationship of obesity to blood estrogen. *Cancer research.* **42**(8):3289-3294.