بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Assessment of Serum Levels of Total Testosterone Among Sudanese Male With Type Two Diabetes Mellitus In Khartoum State.

تقييم مستوى هرمون التستيرون الكلى فى مصل الدم لدى الزكور السودانيون المصابون بمرض السكر من النوع الثانى في ولاية الالخرطوم.

A dissertation submitted for partial fulfillment for the requirements of

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الآيه

بسم الله الرحمن الرحيم

{اللَّهُ نُورُ السَّمَاوَاتِ وَالْأَرْضِ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ الْمِصْبَاحُ فِي زُجَاجَةٍ الزُّجَاجَةُ كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِنْ شَجَرَةٍ مُبَارَكَةٍ زَيْتُونَةٍ لَا شَرْقِيَّةٍ وَلَا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارٌ نُورٌ عَلَى نُورٍ يَهْدِي اللَّهُ لِنُورِهِ مَنْ يَشَاءُ وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ}.

صدق الله العظيم

(سورة النور الايه35)

Dedication

To……..

My mother and father;

For their genuine love

To…….

My dear husband;

For complete help me in every things

To……

My all family members;

For their continues support

To…….

All those who offered their kind assistance:

To……

My all teachers

To…….

All diabetic patients

We dedicate this study

Acknowledgment

Thank you my God, for giving me the ability and courage to complete and bring this research to light.

I want to express my gratefulness and profound gratitude to my supervisor, Dr: Noon for her advice comments and patience bring about the outcome of the dissertation.

Also I would take extend my thanks to the staff of alfoaud hospital and antali center especially nurse Hanna for help me to collect the samples and taking the questionnaire.

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Abstract

Type 2 diabetes is associated with an increased fat mass (in particular central adiposity), reduced insulin sensitivity, impaired glucose tolerance, elevated triglycerides and cholesterol and low HDL-cholesterol. All these factors may be lead to secondary hypogonadism in men and reduced total testosterone level in blood.

This cross-sectional study was carried out to investigate the level of total testosterone among Sudanese male with type 2 diabetes mellitus in Khartoum state. Fifty blood samples were collected from diabetic's male (type2) as case group and fifty blood samples were collected from non diabetic's male as control group from Khartoum state during the period from February to September 2018.All blood samples were collected in two containers which one is plain container and the serum are separated after clot formation for The estimation of serum total testosterone which was done by using automated hormone analyzer (tosoh analyzer),and another containers is EDATA the whole blood was used to estimate HbA1c by clover A1Canalyzer, then the results was analyzed by using SPSS computer program.

Statistical analysis showed a significant decrease in serum total testosterone level in male with type 2diabets mellitus (mean ± SD: 4.62 ± 0.88ng/ml) when compared to control (6.35 ± 0.67ng/ml) with p. value= 0.000.

Results showed there was negative correlation between age of diabetic's males and serum total testosterone level (R= -0.614, P=0.000) ,also there was negative correlation between body mass index of diabetic's males and serum total testosterone level (R= -0.375, P=0.007), also there was strong negative correlation between HbA1c of diabetic's male and serum testosterone level (R= -0.710, P=0.000) , finally there no correlation between duration of diabetes and serum total testosterone level(R= -0.180,P=0.211). .

In conclusion: diabetic's males with type 2 had decreased total testosterone levels which negatively correlated with age, BMI and HbA1c.

مستخلص الدراسة

مرض السكر من النوع التانى يتصف بوجود السمنه خاصة دهون الوسط ،ومقاومه الخلايا لهرمون الانسولين ،

وخلل في تحمل الجسم لجلكوز الدم ، وارتفاع الدهون التلاثية،والكولسترول، وانخفاض الكستورل العالى الكثافه(الكلوسترول الجيد)،كل هذه العوامل ربما تؤدي لاصابه الذكور بقصور الغدد التناسيلة من النوع التانى وبالتالى انخفاض مستوى هرمون التستيرون الكلى في الدم.

اجريت هذه الدراسه لمعرفه تاثير مرض السكرى من النوع التاني على مستوى هرمون التستيرون الكلى بين الذكور السودانيون في ولاية الخرطوم ،تم اختيار50شخصا من الذكور المصابون بمرض السكرى من النوع التانى كفئه دراسه وايضا تم اختيا50شخصا من الذكور غير المصابين كمجموعه ضابطه من ولاية الخرطوم خلال الفترة من قبراير الى سبتمبر 2018 .

كل العينات الدم التى جمعت وضع جزء منها فى انابيب خاليه من موانع التجلط للحصول على سيروم الدم وتحليل مستوى هرمون التسيتسرون الكلى فيه باستخدام جهاز التوسو الالى ،ووضع الجزء الاخر فى انابيب بها مانع التجلط (EDTA) لتحليل مستوى السكر المجلكز باستخدام جهاز الكلوفر.

كل النتائج حللت احصائيا باستخدام الحزمه الاحصائيه للعلوم الاجتماعيه.

اظهر التحليل الاحصائي انخفاضا ذو دلاله احصائية في مستوى هرمون التستيرون الكلي في الدم عند الذكور المصابين بمرض السكر من النوع التاني (4.62 ± 0.88) عند مقارنته بالفئه الضابطه(6.35 ± 0.67) بقيمه احتماليه (0.000).

اظهرت النتائج وجود علاقه ارتباط عكسى بين عمر الذكور المصابين بمرض السكر من النوع التاني ومستوى هرمون التستيرون الكلي في الدم في بقيمه احتماليه (0.000) وقيمه ارتباط (-0.614)، وايضا اظهرت الدراسة علاقه ارتباط عكسى بين معامل كتلةجسم الذكور المصابين بمرض السكرى من النوع التاني ومستوي هرمون التستيرون الكلى في الدم بقيمه احتماليه (0.007) وقيمه ارتباط (-0.375)، وايضا اظهرت الدراسة علاقة ارتباط عكسى قوي بين السكر المجلكز للذكور المصابين بمرض السكرى من النوع التاني ومستوى هرمون التستيرون الكلي فى الدم بقيمه احتماليه (0.000) وقيمه ارتباط (-0.710).واخير اظهرت الدراسة عدم وجود علاقة بين فترة مرض السكرى ومستوي هرمون التستيرون الكلى فى الدم بقيمه احتماليه (0.211) وقيمه ارتباط (-0.180).

خلصت هذه الدراسه ان مستوى هرمون التستيرون الكلى فى الدم ينخفض لدى الذكور المصابين بمرض السكرى من النوع التاني ،مع وجود علاقة ارتباط عكسي مع عمر ،كتلة معامل الجسم والسكر المجلكز.

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List of abbreviation

BMI	body mass index
DM	diabetes mellitus
DTH	Di hydro testosterone
ELISA	enzyme linked immune sorbent assay
FSH	follicular stimulating hormone
GLUT	Glucose transporter
GnRH	gonadotrophic releasing hormone
HBA1C	glycated hemoglobin
HLA	human leukocyte antigen
HPA	hypothalamic –pituitary-adrenal axis
HRP	histidin rich protein
ICA	islet cell anti bodies
LADA	Latent autoimmune diabetes in adult
LED	light emitting diode
LH	luteinizing hormone
MODY	maturity onset diabetes of the young
NIDDM	Non insulin dependent diabetes mellitus
NGSP	National Glycohemoglobin standardization program
PD	photo diode
SHBG	Sex hormone binding globulin

Chapter one

1. Introduction, Rationale, objectives

1.1. Introduction:

Type 2 diabetes mellitus, formerly non-insulin dependent diabetes mellitus or adult onset diabetes, is a metabolic disorder that is characterized by hyperglycemia in the context of insulin resistance and relative insulin deficiency (vinay etal .,1999). Over 90% of people with diabetes mellitus are type 2 diabetics and it is reported to be associated with certain endocrine disorders, in particular hypogonadism in men (Burtis etal .,2008).Testosterone is a hormone that plays a key role in carbohydrate, fat and protein metabolism. It has been known for some time that testosterone has a major influence on body fat composition and muscle mass in the male. Testosterone deficiency is associated with an increased fat mass (in particular central adiposity), reduced insulin sensitivity, impaired glucose tolerance, elevated triglycerides and cholesterol and low HDL-cholesterol. All these factors are found in the metabolic syndrome (Mets) and type2 diabetes, contributing to Cardiovascular risk (Daniel,2013).

1.2. Rational:

A number of epidemiological studies have suggested an association of obesity, metabolic syndrome, and dysglycemia with low serum testosterone and poor quality of life in type 2 diabetes (Beatrice etal., 2014).This association is of clinical significance because low total testosterone in men has been reported to be associated with increased cardiometabolic risk factor burden, including a greater prevalence of dyslipidemia and atherosclerosis, and an overall increase in mortality. To the best of our knowledge few published data are found regarding assessment of levels of total Testosterone among Sudanese male with type 2 diabetes mellitus that's way we attempt to do this study.

1.3. Objectives:

1.3.1. General objective

To assess the level of total testosterone among Sudanese patients with type 2 diabetes mellitus.

1.3.2. Specific objectives:

1-To estimate and compare the level of total testosterone in Sudanese patients with type 2 diabetes mellitus, and healthy individuals.

2-To correlate between the level of total testosterone and the HbA1C in patient with type 2 diabetes mellitus.

3-To correlate between the level of total testosterone and the body mass index in patient with type 2 diabetes mellitus.

4- To correlate between the level of total testosterone and age in patient with type 2 diabetes mellitus.

5-To find correlation between the level of total testosterone and duration of diabetes mellitus.

Chapter two

2-literature review:

2.1. Diabetes mellitus:

2.1.1. Definition:

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both. Insulin deficiency in turn leads to chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism (Kumar, 2002; Beverly, 2003). Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced (Shoback etal., 2011).

2.1.2. Classification of diabetes mellitus

Diabetes mellitus is classified into four broad categories : type 1, type 2 , gestational diabetes , and "other specific types the "other specific types" are a collection of a few dozen individual causes (Shoback etal., 2011).

Type 1 Diabetes

- β-cell destruction which leads to absolute insulin deficiency
- Usually mediated by immune mechanisms
- LADA (latent autoimmune diabetes in adults) is classified as type 1 diabetes (kerner and Brukel, 2014).

Type 2diabetes

- Can range from predominant insulin resistance with relative insulin deficiency to prevailing defective secretion with insulin resistance.
- Is frequently associated with other problems of the so called metabolic syndrome (kerner and, Brukel 2014).

Other Specific Diabetes Type

- Diseases of the exocrine pancreas (e. g. pancreatitis,

Cystic fibrosis, Hemochromatosis).

- Endocrinopathies (e. g. Cushing syndrome, acromegaly,

Pheochromocytoma).

- Drug induced (e. g. Glucocorticoids, neuroleptics, Alpha-interferon's, pentamidine).
- Genetic defects of the β-cell function (e. g.MODY forms).
- Genetic defects of insulin action
- Other genetic syndromes which can be associated

With diabetes.

- ❖ Infections
- ❖ Rare forms of auto-immune mediated diabetes

Gestational Diabetes

Glucose tolerance impairments that first appear or are first diagnosed during pregnancy (kerner and Brukel, 2014).

2.1.2.1. Type2 Diabetes mellitus (NIDDM):

Type 2 diabetes is the predominant form of diabetes and accounts for at least 90% of all cases of diabetes mellitus (Gonzalez etal., 2009).

The incidence of diabetes increases with age, with most cases being diagnosed after the age of 40 years. This equates to a lifetime risk of developing diabetes of 1 in 10 (Neil etal., 1987). Type 2 diabetes is a heterogeneous disorder caused by a combination of genetic factors related to impaired insulin secretion, insulin resistance and environmental factors such as obesity, over eating, lack of exercise, and stress as well as aging (Kaku, 2010). It is typically a multifactorial disease involving multiple genes and environmental factors to varying extents (Holt, 2004).

Pathogenesis of type 2 diabetes:

Under normal physiological conditions, plasma glucose concentrations are maintained within a narrow range, despite wide fluctuations in supply and demand, through a tightly regulated and dynamic interaction between tissue sensitivity to insulin (especially in liver) and insulin secretion (DeFronzo and ferraannini, 1988). In type 2 diabetes these mechanisms break down, with the consequence that the two main pathological defects in type 2diabetes are impaired insulin secretion through a dysfunction of the pancreatic β-cell, and impaired insulin action through insulin resistance (Holt, 2004).

Type 2 diabetes mellitus has a greater genetic association than type 1DM, the pathogenesis of type 2 diabetes mellitus is characterized by impaired insulin secretion and insulin resistance as shown in Figure 2. T he 100% concordance rate in identical twins is thought to be over-estimated, due to a selection or reporting bias. A population based twin study in Finland has shown a concordance rate of 40%, and environmental effect may a possible reason for the higher concordance rate for type 2 diabetes mellitus than for type 1 diabetes mellitus (Kaprio et al., 1992). Type 2 diabetes mellitus affects 1 to 2% of Caucasians (Cook et al., 1993) but it is much

higher in some ethnic groups such as Pima Indians (Knowler et al., 1993) and Arabs (Richens et al., 1988) and approaches 50% in South India. This indicates that genetic factors are more important than environmental factors. Except for maturity onset diabetes of the young (MODY), the mode of inheritance for type 2 diabetes mellitus is unclear. MODY, inherited as an autosomal dominant trait, may result from mutations in glucokinase gene on chromosome 7p. Glucokinase is a key enzyme of glucose metabolism in beta cells and the liver (Froguel et al., 1993; Hattersley et al., 1992). MODY is defined as hyperglycemia diagnosed before the age of twenty-five years and treatable for over five years without insulin in cases where islet cell antibodies (ICA) are negative and HLA-DR3 and DR4 are heterozygous.

Insulin resistance by itself may be a secondary event in type 2 DM, since it is also found in non-diabetic obese individuals. Insulin secretion defect may be the primary event, presenting as impaired pulsattile secretion of insulin. Hence, hyperglycemia is an inducer as well as a consequence of impaired islet cell function and insulin resistance. Many factors contribute to the insulin insensitivity including obesity and its duration (Evephart et al., 1992), age, lack of exercise, increased dietary fat and decreased fibers and genetic factors.

Insulin resistance in type 2 DM is not totally clear, it may involve reduced insulin receptor number, it may be secondary to hyperinsulinemia and hyperglycemia, (Vuorinen-Markkola et al., 1992) or it may result from reduced tyrosine kinase activity (Comi et al., 1987; Bonadonna et al., 1993; Sten-linder et al., 1993**)** even abnormalities distal to the receptor involving glucose transporter proteins through a family of glucose transporter genes (Mueckler, 1990). The GLUT2 gene, expressed in liver and pancreatic beta cells, and GLUT4, expressed in skeletal muscle and adipocytes, are strong candidate genes for the genetic susceptibility to type 2 DM. Analysis of these two glucose transporter genes, in addition to GLUT1, encoding for the brain/erythrocyte glucose transporter, has yielded, in Caucasians, no association of any RFLP marker on haplotype with either type 2 DM or obesity (Oelbaum, 1992).

Obesity has genetic as well as environmental causes. It has a strong effect on the development of type 2 DM (Bjorntorp, 1992; Haffner et al., 1992) as it is found in Western countries (NDDG, 1979; Wilson et al., 1981) and some ethnic groups such as Pima Indians (Joffe et al., 1992; Knowler et al., 1993)**.**

Environmental factors in the pathogenesis of type 2 diabetes:

Aging, obesity, insufficient energy consumption, drinking, smoking, etc are independent risk factors of pathogenesis of type 2 diabetes. Obesity (particularly visceral fat obesity) due to a lack of exercise is accompanied by a decrease in muscle mass, induces insulin resistance, and is closely associated with the rapid increase in the number of middle and high aged patients. The changes in dietary energy sources, particularly the increase in fat intake, the decrease in starch intake, the increase in the consumption of simple sugars, and the decrease in dietary fiber intake, contribute to obesity and cause deterioration of glucose tolerance. Even mild obesity (Body mass index (BMI) < 25) causes a 4 to 5 fold increase in risk of developing diabetes, if accompanied by the increase in visceral fat mass. People prone to visceral fat accumulation due to hyper alimentation, and risk factors for diabetes are linked to the accumulation of visceral fat.

Pathophysiology of type 2 diabetes (NIDDM):

Individuals with NIDDM have detectable levels of circulating insulin, unlike patients with IDDM and the pathophysiology of type2 diabetes is described in Figure 3. On the basis of oral glucose tolerance testing the essential elements of NIDDM can be divided into four distinct groups:

I) those with normal glucose tolerance.

ii) Chemical diabetes (called impaired glucose tolerance).

iii) Diabetes with minimal fasting hyperglycemia (fasting plasma glucose less than 140 mg/dl).

iv) Diabetes mellitus in association with overt fasting hyperglycemia (fasting plasma glucose greater than 140 mg/dl).

The individuals with impaired glucose tolerance have hyperglycemia in spite of having highest levels of plasma insulin, indicating that they are resistant to the action of insulin. In the progression from impaired glucose tolerance to diabetes mellitus, the level of insulin declines indicating that patients with NIDDM have decreased insulin secretion. Insulin resistance and insulin deficiency are common in the average NIDDM patients (Holt, 2004). Insulin resistance is the primary cause of NIDDM, however some researcher contend that insulin deficiency is the primary cause because a moderate degree of insulin resistance is not sufficient to cause NIDDM (Raju and Raju, 2010). Most patients with the common form of NIDDM have both defects. Recent evidence has demonstrated a role for a member of the nuclear hormone

receptor super family of proteins in the etiology of type 2 diabetes (Raju and Raju, 2010).

2.1.3.Complications of diabetes mellitus:

All forms of diabetes increase the risk of long-term complications. These typically develop after many years (10–20), but may be the first symptom in those who have otherwise not received a diagnosis before that time.

The major long-term complications relate to damage to blood vessels. Diabetes doubles the risk of cardiovascular disease (Sarwar etal .,2010) and about 75% of deaths in diabetics are due to coronary artery disease (OGara .,etal 2013) other "macro vascular" diseases are stroke, and peripheral vascular disease.

The primary complications of diabetes due to damage in small blood vessels include damage to the eyes, kidneys, and nerves (WHO, 2014). Damage to the eyes, known as diabetic retinopathy, is caused by damage to the blood vessels in the retina of the eye, and can result in gradual vision loss and blindness (WHO, 2014). Damage to the kidneys, known as diabetic nephropathy, can lead to tissue scarring, urine protein loss, and eventually chronic kidney disease, sometimes requiring dialysis or kidney transplant(WHO,2014).Damage to the nerves of the body, known as diabetic neuropathy, is the most common complication of diabetes. The symptoms can include numbness, tingling, pain, and altered pain sensation, which can lead to damage to the skin. Diabetes-related foot problems (such as diabetic foot ulcers) may occur, and can be difficult to treat, occasionally requiring amputation. Additionally, proximal diabetic neuropathy causes painful muscle wasting and weakness (WHO, 2014).

2.2. Glycated hemoglobin:

Glycated hemoglobin (hemoglobin A1C, HbA_{1c}, A1C, or Hb_{1c}; sometimes also HbA1c or HGBA1C) is a form of hemoglobin that is measured primarily to identify the three month average plasma glucose concentration. The test is limited to a three month average because the lifespan of a red blood cell is three months HbA1c is formed in two steps by the nonenzymatic glycation of HbA. The First step is the formation of an unstable aldimine (labile A1C, or pre-A1c), a reversible reaction between the carbonyl group of glucose and the N terminal valine of the β-chain of hemoglobin. Labile A1C formation is directly proportional to the blood glucose concentration. During red blood cell circulation, some of the labile A1C is converted (Amadori rearrangement) to form a stable ketoamine, HbA1c (Mayer1,983). Normal levels of glucose produce a normal amount of glycated hemoglobin. As the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases in a predictable way. This serves as a marker for average blood glucose levels over the previous three months before the measurement as this is the lifespan of red blood cells. In diabetes mellitus, higher amounts of glycated hemoglobin, indicating poor control of blood glucose levels, have been associated with disease nephropathy, neuropathy, and retinopathy. Monitoring HbA_{1c} in diabetic patients, for the purpose of assessing glycemic control and modifying therapy, may improve outcomes. (Mayer,1983)

2.3. Testosterone:

2.3.1. Definition:

Testosterone is steroid hormone from androgen group and is found in human and other vertebrate. In human and other mammals, testosterone is secreted primarily by the testicles of males, and to lesser extent the ovaries of females, small amounts are also secreted by the adrenal glands testosterone it is principle male sex hormone and anabolic steroid (Mooradian, 1987).

2.3.2. Biochemistry of testosterone:

Biosynthesis:

The largest amount of testosterone (>95%) are produced by the testes in men (Mooradian, 1987).It is also synthesized in far small quantities in women by the thecal cell of ovaries, by the placenta, as well as by the zone reticularies of the adrenals cortex and even skin in both sexes (Zouboulis and degitz, 2004).The male testicular androgen are synthesized in the interstitial tissue of the leyding cell after LH binding to receptor on the plasma membrane Of the leyding cell (praful, 2003)Like other hormone testosterone is derived from cholesterol (Waterman andKeeney,1992).The first step in biosynthesis involve the oxidation cleavage of side chain of cholesterol by CYP17A enzyme, mitochondrial cytochrome P450 oxidase with loss of six carbon atoms to give pregnenolone In the next step, two additional carbon atoms are removed by the CYP17A enzyme in the endoplasmic reticulum to yield variety of C19 steroids (Zuber etal.,1986).in addition the 3-hydroxyl group is oxidized by3-β-hsd to produce androstenedione, in the final step, the C-17 ketogroup androstenedione is reduced by 17-β-hydroxy steroid dehydrogenase to yield testosterone.

Metabolism of testosterone:

98% of testosterone in plasma is bound to protein .65% is bound to beta Globulin called sex hormone binding globulin (SHBG) and 33% to albumin (Ganong,2012) And the rest is about 2-3% is free testosterone which was thought to be Represent biologically active fraction, Approximately 0.3% of testosterone is converted to estradiol by aromatase CYP19A1 (Meinhard, 2002) . But most of testosterone is converted to 17-keto steroids. Principally androsterone and it is isomer etio-cholanoloneand excreted in urine (Ganong,2012). Approximately 7% of testosterone is reduced to 5α-dihydrotestosterone (DTH) by the cytochrome P450 enzyme 5α-reducatase (Randall, 1994).An enzyme is highly expressed in male sex organ and hair

follicles (Mooradian ,1987). While estradiol has completely Different activities (feminization) compared to testosterone (masculinization), also testosterone and DTH may be deactivated or cleared by enzymes that hydroxyl ate at the 6,7,15 or16 position (Trager, 1977).

2.3.3. Regulation of testosterone level in blood:

In males testosterone is synthesized primarily in leyding cells, the number of leyding cells is turn is regulated by LH and FSH; in addition the amount of testosterone produced by existing leyding cell is under the control of LH ,which is regulates the expression of 17-βhydroxy steroid dehydrogenase(Payne and O'Shaughness,1996).

The amount of testosterone synthesized is regulated by the hypothalamic- Pituitary–testicular axis (Swerdloffetal.,1992).

When testosterone level is low, GnRH is released by the hypothalamus Which is turn stimulate the pituitary gland to release FSH and LH, these later two hormones stimulate the testis to synthesize testosterone, finally increasing levels of testosterone through negative feedback mechanism act on hypothalamus and pituitary to inhibit the release of GnRH and FSH /LH respectively.

2.3.4. Health effects of testosterone:

In general, androgens promote protein synthesis and growth of those tissues with androgen receptors(Sheffield Moore, 2000) Testosterone effects can be classified as virilizing and anabolic, though the distinction is somewhat artificial, as many of the effects can be considered both.

- **Anabolic effects** include growth of muscle mass and strength, increased bone density and strength, and stimulation of linear growth and bone maturation (HandeIsman, 2013).
- **Androgenic effects** include maturation of the sex organs, particularly the penis and the formation of the scrotum in the fetus, and after birth (usually at puberty) a deepening of the voice, growth of the beard and auxiliary hair. Many of these fall into the category of male secondary sex characteristics.

Testosterone effects can also be classified by the age of usual occurrence. For postnatal effects in both males and females, these are mostly dependent on the levels and duration of circulating free testosterone.

Before birth

The prenatal androgen effects occur during two different stages. Between 4 and 6 weeks of the gestation.

- Genital virilization (midline fusion, phallic urethra, scrotal thinning and rugation, phallic enlargement); although the role of testosterone is far smaller than that of dihydrotestosterone.
- Development of prostate and seminal vesicles.

During the second trimester, androgen level is associated with gender formation (Swaab and Garcia,2009) this period affects the femininization or masculinization of the fetus and can be a better predictor of feminine or masculine behaviors such as sex typed behavior than an adult's own levels. A mother's testosterone level during pregnancy is correlated with her daughter's sex-typical behavior as an adult, and the correlation is even stronger than with the daughter's own adult testosterone level (Browne, 2002).

Early infancy

Early infancy androgen effects are the least understood. In the first weeks of life for male infants, testosterone levels rise. The levels remain in a pubertal range for a few months, but usually reach the barely detectable levels of childhood by 4–6 months of age (Forest etal,. 1973) (Corbier etal,. 1992).

The function of this rise in humans is unknown. It has been speculated that "brain masculinization" is occurring since no significant changes have been identified in other parts of the body(Dakin etal,.2008) The male brain is masculinized by the aromatization of testosterone into estrogen, which crosses the blood–brain barrier and enters the male brain, whereas female fetuses have alpha-fetoprotein, which binds the estrogen so that female brains are not affected(Kalat ,2009).

Pre-peripubertal:

Pre- Peripubertal effects are the first observable effects of rising androgen levels at the end of childhood, occurring in both boys and girls.

- Adult-type body odor
- Increased oiliness of skin and hair, acne
- Pubarche (appearance of pubic hair)
- Auxiliary hair
- Growth spurt, accelerated bone maturation
- Hair on upper lip, on chin, and growth of sideburns (pinyerd and Zipf, 2005**).**

Pubertal

Pubertal effects begin to occur when androgen has been higher than normal adult female levels for months or years. In males, these are usual late pubertal effects, and

occur in women after prolonged periods of heightened levels of free testosterone in the blood.

- Enlargement of sebaceous glands. This might cause acne.
- Penis or clitoris enlargement (Ganong, 2012).
- Increased libido and frequency of erection or clitoral engorgement
- Pubic hair extends to thighs and up toward umbilicus
- Facial hair (sideburns, beard, moustache)
- Loss of scalp hair (Androgenetic alopecia)
- Chest hair, periareolar hair, perianal hair
- Leg hair, armpit hair
- Subcutaneous fat in face decreases
- Increased muscle strength and mass(Bhasin etal .,1996)
- Deepening of voice
- Growth of the Adam's apple
- Growth of spermatogenic tissue in testicles, male fertility
- Growth of jaw, brow, chin, nose, and remodeling of facial bone contours, in conjunction with human growth hormone
- Shoulders become broader and rib cage expands
- Completion of bone maturation and termination of growth. This occurs indirectly via estradiol metabolites and hence more gradually in men than women.
- Mental: More aggressive, active attitude. Interest in sex develops.
- Skin: Sebaceous gland secretion thickens and increases (predisposing to acne). (Ganong,2012).

Adult

Adult testosterone effects are more clearly demonstrable in males than in females, but are likely important to both sexes. Some of these effects may decline as testosterone levels decrease in the later decades of adult life (Kelsey etal., 2014).

2.3.5. Biological uses of testosterone:

- Testosterone is necessary for normal sperm development. It activates genes in Sertoli cells, which promote differentiation of spermatogonia.
- Regulates acute HPA (Hypothalamic–pituitary–adrenal axis) response under dominance challenge (Mehta etal., 2008).
- Regulator of cognitive and physical energy
- Maintenance of muscle trophism

- Testosterone regulates the population of thromboxane A_2 receptors on megakaryocytes and platelets and hence platelet aggregation in humans (Ajayi and Halushka, 2005) (Ajayi etal., 1995).
- High androgen levels are associated with menstrual cycle irregularities in both clinical populations and healthy women (Vananders and watson, 2006).

2.3.6 Male hypogonadism:

Hypogonadism is a medical term for decreased functional activity of the gonads. The gonads (ovaries or testes) produce hormones (testosterone, estradiol, anti mullerian hormone, progesterone, inhibin B, activin) and gametes (eggs or sperm) Male hypogonadism is characterized by a deficiency in testosterone – a critical hormone for sexual, cognitive, and body function and development. Clinically low testosterone levels can lead to the absence of secondary sex characteristics, infertility, muscle wasting, and other abnormalities. Low testosterone levels may be due to testicular, hypothalamic, or pituitary abnormalities. In individuals who also present with clinical signs and symptoms, clinical guidelines recommend treatment with testosterone replacement therapy (YiaIamas and Hayes, 2003).

Classification OF male Hypogonadism:

There are two basic types of hypogonadism that exist:

Primary: This type of hypogonadism – also known as primary testicular failure – originates from a problem in the testicles.

Secondary: This type of hypogonadism indicates a problem in the hypothalamus or the pituitary gland – parts of the brain that signal the testicles to produce testosterone. The hypothalamus produces the gonadotropin releasing hormone, which signals the pituitary gland to make the follicle-stimulating hormone (FSH) and luteinizing hormone. The luteinizing hormone then signals the testes to produce testosterone. Either type of hypogonadism may be caused by an inherited (congenital) trait or something that happens later in life (acquired), such as an injury or an infection.

Primary Hypogonadism

Common causes of primary hypogonadism include:

Klinefelter's Syndrome: This condition results from a congenital abnormality of the sex chromosomes, X and Y. A male normally has one X and one Y chromosome. In Klinefelter's syndrome, two or more X chromosomes are present in addition to one Y chromosome. The Y chromosome contains the genetic material that determines the sex of a child and the related development. The extra X chromosome that occurs in Klinefelter's syndrome causes abnormal development of the testicles, which in turn results in the underproduction of testosterone

Undescended testicles

Before birth, the testicles develop inside the abdomen and normally move down into their permanent place in the scrotum. Sometimes, one or both of the testicles may not descend at birth. This condition often corrects itself within the first few years of life without treatment. If not corrected in early childhood, it may lead to malfunction of the testicles and reduced production of testosterone.

Mumps orchitis

If a mumps infection involving the testicles in addition to the salivary glands (mumps orchitis) occurs during adolescence or adulthood, long-term testicular damage may occur. This may affect normal testicular function and testosterone production.

Hemochromatosis

Too much iron in the blood can cause testicular failure or pituitary gland dysfunction, affecting testosterone production.

Injury to the Testicles

Because of their location outside the abdomen, the testicles are prone to injury. Damage to normally developed testicles can cause hypogonadism. Damage to one testicle may not impair testosterone production.

Cancer treatment

Chemotherapy or radiation therapy for the treatment of cancer can interfere with testosterone and sperm production. The effects of both treatments are often temporary, but permanent infertility may occur. Although many men regain their fertility within a few months after the treatment ends, preserving sperm before starting cancer therapy is an option that many men consider. Howell et al. reported that hypogonadism was seen in 30% of the men with cancer and 90% of these gentlemen had germinal epithelial failure (Howell etal., 1999).

Normal aging

Older men generally have lower testosterone levels than younger men do. As men age, there's a slow and continuous decrease in testosterone production. The rate that testosterone declines varies greatly among men. As many as 30% of men older than 75 have a testosterone level that is below normal, according to the American Association of Clinical Endocrinologists. Whether or not treatment is necessary remains a matter of debate (Harman, 2001).

Secondary Hypogonadism

In secondary hypogonadism, the testicles are normal, but function improperly due to a problem with the pituitary or hypothalamus. A number of conditions can cause secondary hypogonadism, including:

Kallmann syndrome

Abnormal development of the hypothalamus – the area of the brain that controls the secretion of pituitary hormones – can cause hypogonadism. This abnormality is also associated with the impaired development of the ability to smell (anosmia).

Pituitary disorders

An abnormality in the pituitary gland can impair the release of hormones from the pituitary gland to the testicles, affecting normal testosterone production. A pituitary tumor or other type of brain tumor located near the pituitary gland may cause testosterone or other hormone deficiencies. Also, the treatment for a brain tumor such

as surgery or radiation therapy may impair pituitary function and cause hypogonadism.

Inflammatory disease

Certain inflammatory diseases such as sarcoidosis, Histiocytosis, and tuberculosis involve the hypothalmus and pituitary gland and can affect testosterone production, causing hypogonadism.

HIV/AIDS

This virus can cause low levels of testosterone by affecting the hypothalamus, the pituitary, and the testes.

Medications

The use of certain drugs, such as, opiate pain medications and some hormones, can affect testosterone production (Daniell , 2002).

Obesity

Being significantly overweight at any age may be linked to hypogonadism.

Stress-induced Hypogonadism

Stress, excessive physical activity, and weight loss have all been associated with hypogonadism. Some have attributed this to stress-induced hypercortisolism, which would suppress hypothalamic function (Cumming etal., 1983).

Other condition associated with male hypogonadism:

Chronic illness

Diabetes mellitus

Chronic renal failure

Cancer cachexia

Corticosteroid use Rheumatoid arthritis (Charlesfiman , 2012).

Chapter three

3. Materials and methods

3.1. Materials:

3.1.1. Study design:

Analytical cross sectional study.

3.1.2. Study area:

The study was conducted in Khartoum state in different hospitals and diabetic center.

3.1.3 Study period:

The study was carried during the period from february2018 to september2018.

3.1.4. Study population:

The study was conducted on 50 Sudanese male with type 2 diabetes Mellitus with age group of 32-82 years old (as test group) and apparently healthy individuals (as control group).

3.1.5. Criteria for selection the cases:

Inclusion criteria:

The male with type 2 diabetes mellitus was included in the study.

Exclusion criteria:

Any patients with type1diabetes mellitus, renal failure, thyroid disorder, adrenal disorder, prostate disorder were excluded from study.

3.1.6 Ethical consideration:

The study was approved by the scientific committee of clinical chemistry department College of medical laboratory science of Sudan University of science and technology, then an informed consent was obtained from all participants. (appendix1)

3.1.7 Data collection:

Questionnaire was specifically designed to obtain information which helps in the study. (appendix II)

3.1.8. Sampling:

After informed consent, and the use of local antiseptic for the skin (70% ethanol) a sample of venous blood (about 5ml) was collected from each volunteers included in the study from the arm, and poured into two containers one container is EDTA tube for direct HbA1c estimation and other container is plain for serum preparation, serum was separated after clot retraction by centrifugation (at3000rpm for 5mint) and stored at -20˚c until analysis of testosterone.

3.2. Methods:

3.2.1. Measurement of HbA1C:

Principle:

The clover A1C system is fully automated boronate affinity assay for the determination of the percentage of hemoglobin A1C (HbA1C%) in whole blood. the test cartridge is composed of cartridge and reagent pack, the blood sample(about4µ) is collected at the sample collecting area on the reagent pack , which is pre-filled with reagent solution and rinsing solution ,the reagent solution contains agents that lyses erythrocytes and bind hemoglobin specially ,as well as boronate resin that binds cis-diols of glycated hemoglobin, then the reagent pack has been inserted into clover A1C analyzer(in which the cartridge has been placed) .the cartridge is automatically rotated ,placing the blood sample in the measuring zone ,the total hemoglobin is photometrically measured by the diffused reflectance of the optical sensor composed of both LED and PD. Then assembled cartridge is rotated and the rinsing solution washes out non- glycated hemoglobin from the blood sample, enabling photometrical measurement of glycated hemoglobin.

The ratio of glycated hemoglobin and total hemoglobin is calculated.

$$\text{HbA1C\%} = A + \left\lceil \frac{\text{HbA1C}}{totalhemoglobin} * 100 \right\rceil + B$$ (appendix III).

3.2.2. Measurement of total Testosterone level:

ELISA method was used for total testosterone measurement.

Principle:

The testosterone EIA is based on the principle of competitive binding between testosterone in the test specimen and testosterone –HRP conjugate for constant amount of rabbit anti –testosterone .goat anti rabbit IgG-coated well incubated with specimen testosterone ,testosterone HRP-conjugate and rabbit anti- testosterone. During incubation affixed amount of HRP-labeled testosterone competes with the testosterone on the sample for fixed number of binding sites of the specific testosterone antibody, thus the amount of testosterone HRP-conjugate immunologically bound to well progressively decreases as the concentration of testosterone in the specimen increases .unbound labeled testosterone is then removed ,the well is washed and incubated with substrate solution ,measurement were performed spectrophotometically at450nm after stopping the reaction by acidic solution. The intensity of color is directly proportional with enzyme concentration and inversely with hormone concentration. (appendix IV)

3.2.3. Measurement of body mass index:

The following anthropometric parameters were taken into consideration while characterized the subject are obese.

Weight and height:

Weight was recorded to the nearest kilogram (Kg) with subject standing on the weighing machine without shoes. The same weighing machine was used for all participants and the machine was tested with known set of weights for any errors height was recorded with subject erect, bare footed, feet together, back and heels against the upright bar of the height scale .The height measuring equipment consist of vertical bar with a steel tape attached .Attached perpendicularly to the vertical bar was a horizontal bar which was brought down snugly on the examinees head. Weight was recorded in (Kg) whereas height was recorded in (cm).

Body mass index:

Body mass index was calculated from the formula;

BMI= weight in kilogram / (square of height in meters).

3.3. Quality control:

Commercial Control sera (normal and abnormal level) and calibrators were runned with testosterone analysis to ensure accuracy of results, the clover A1Cdaily check cartridge was runned withA1C analysis.

3.4. Data analysis:

Data was statistical analyzed by using computer SPSS program, independent t-test was used for comparison and person's correlation test was used to find correlation.

p-value <0.05was considered significant.

Chapter four

4. Results

Fifty Sudanese males with type 2 diabetes mellitus(with age group32-82 years old) were enrolled in this study to assess the influence of type2 diabetes disease on testosterone level, And fifty Sudanese male without diabetes were served as control group. Data were analyzed statistically using computer program and the results were as follow:

Table (4-1): show base line characteristics of the variables in the study.

Table (4-2): show a significant decrease in total testosterone level in male with type 2 diabetes mellitus (mean±SD: 4.62 ±0.88) when compared to control group (6.35±0.67).

Fig (4-1): there was negative correlation between testosterone level (ng/ml) and age (years) among case group(R=-0.614, P=0.000).

Fig (4-2): there was negative correlation between testosterone level (ng/ml) and BMI (kg/m2) among case group(R=-0.375, P=0.007).

Fig (4-3): there was negative correlation between testosterone level (ng/ml) and HBA1C (g/dl) among case group(R=-0.170, P=0.000).

Fig (4-4): there was no correlation between testosterone level (ng/ml) and duration of disease (years) among case group(R=-0.180,P=0.211).

Table (4-1): base line characteristics of the variables in the study.

Variables	**Value(mean ± SE)**	**Sample range**
BMI(kg/m²)	(29.4 ± 0.62)	18.8-40.3
Age(years)	(55.90 ± 1.81)	32-80
Duration of disease(years)	(8.0 ± 0.66)	1.0-20
HbA1C(g/dl)	(8.3 ± 0.27)	4.8-11.50
Testosterone level in case (ng/ml)	(4.6 ± 0.12)	2.5-6.4
testosterone level in control(ng/ml)	(6.3 ± 0.09)	6.3-8.50

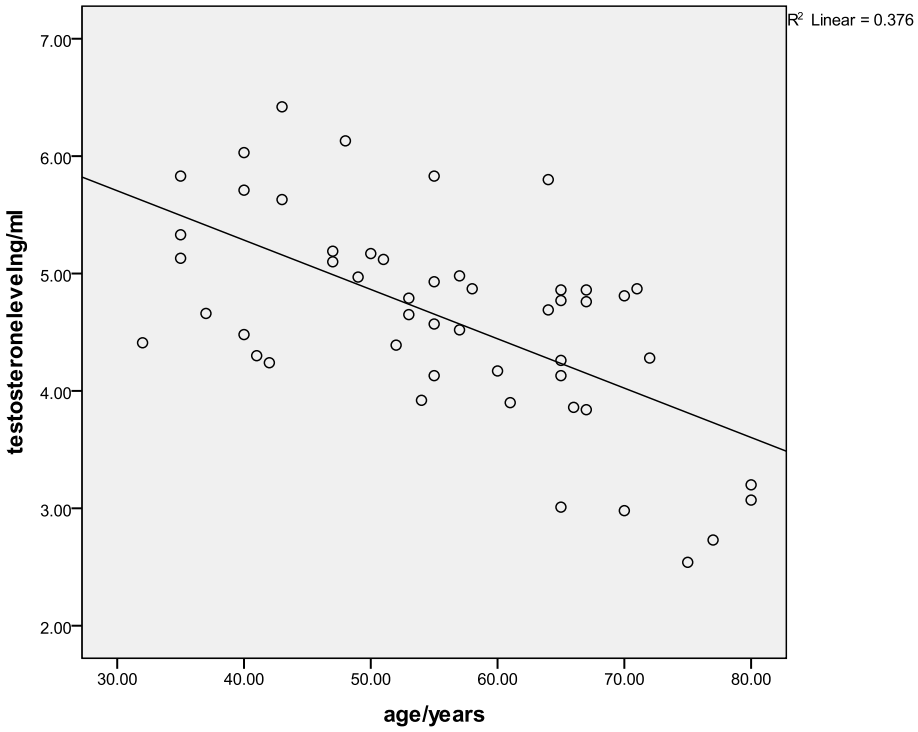
SE=systematic error of mean

.

Table (4-2): comparison between the mean concentrations of the testosterone level in case group and control group.

Variable	N	mean±sd	p-value
Testosterone level (ng/ml)	50 50	Control 6.35± 0.67 Case 4.62 ±0.88	0.000

The independent sample T- test was used, P-value <0.05 considered significant



R=-0.614

P=0.000

Fig (4-1): correlation between total testosterone level (ng/ml) and age (years) among case group.

-R: show the regression of coefficient.

-P: show the strength and significance of correlation.

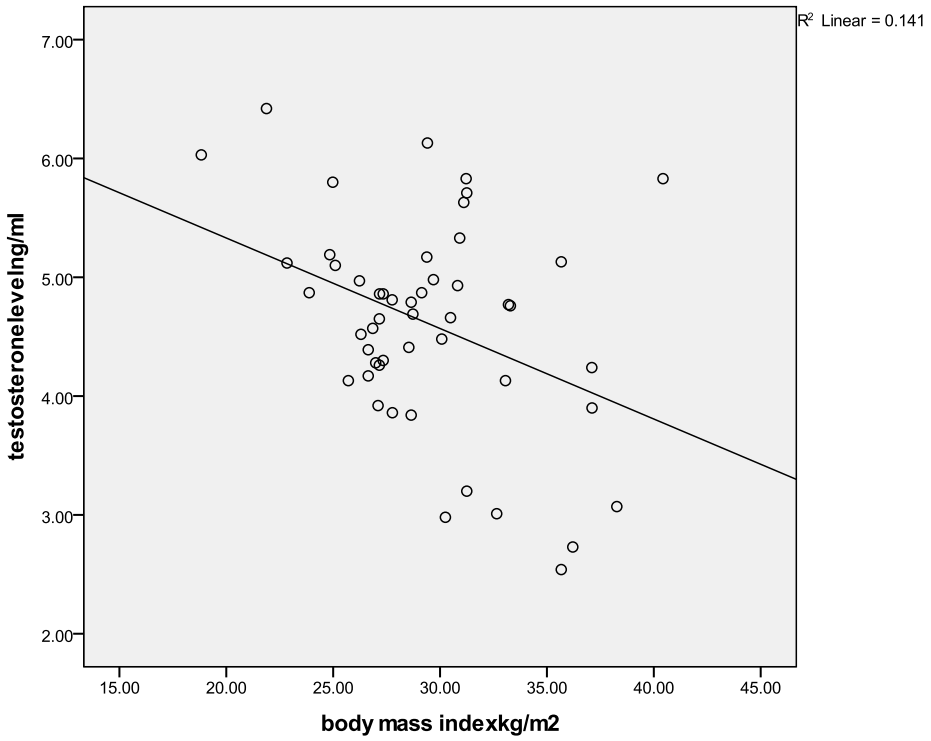


Fig (4-2): correlation between testosterone level (ng/ml) and BMI (kg/m2) among case group(r=-0.375, pvalue=0.007).

-R show the regression of coefficient.

-P : show the strength and significance of correlation.

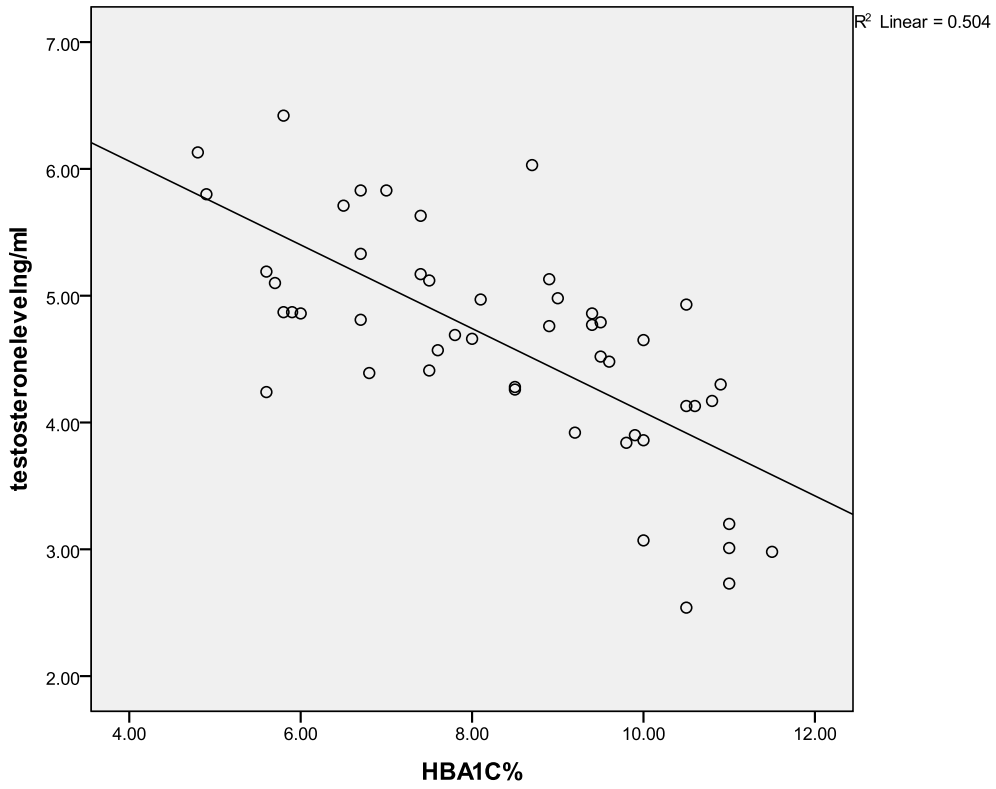


Fig (4-3): correlation between testosterone level (ng/ml) and HBA1C (g/dl) among case group (r=-0.710, p.value=0.000).

-R show the regression of coefficient.

-P : show the strength and significance of correlation.

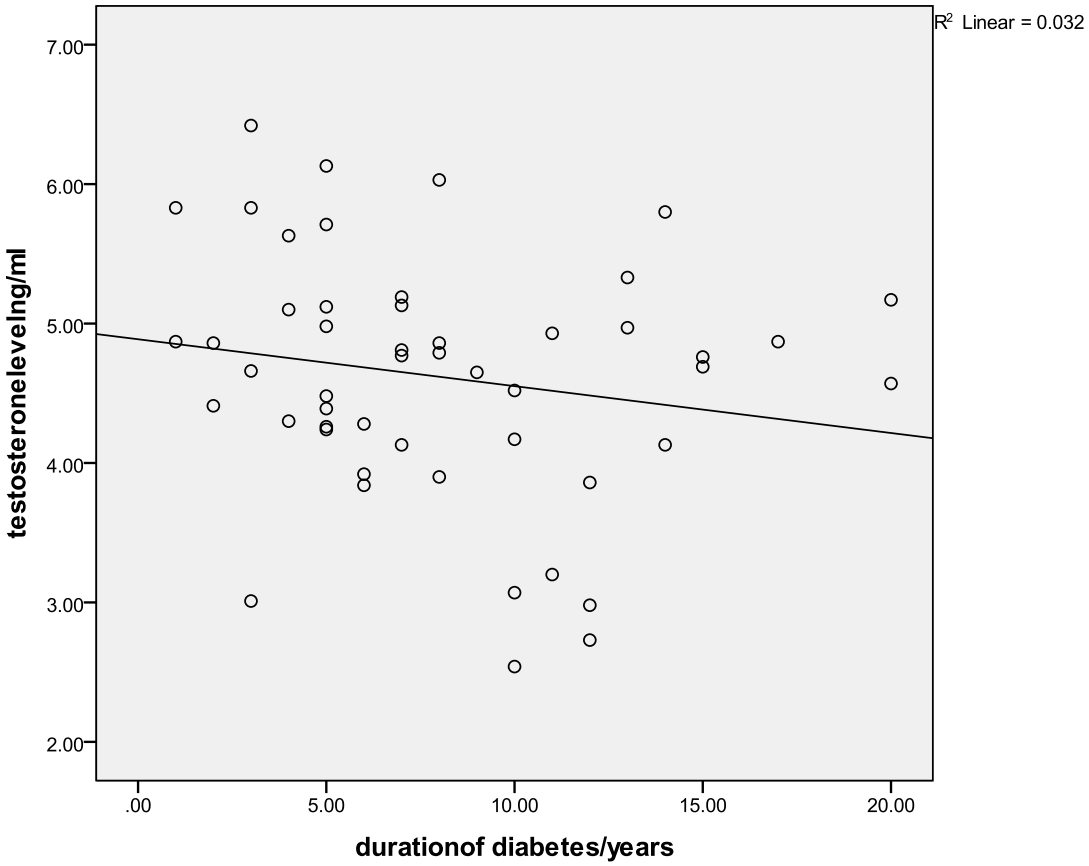


Fig (4-4): correlation between testosterone level (ng/ml) and duration of disease (years) among case group(r=-0.180,p.value=0.211).

-R: show the regression of coefficient.

-P: show the strength and significance of correlation.

Chapter five

5. Discussion, Conclusion & Recommendations:

5.1. Discussion:

Diabetes is the most common endocrine disorder and by the Year 2010, it is estimated that more than 200 million people Worldwide will have DM and 300 million will subsequently have the disease by 2025. And Type 2 diabetes mellitus has been shown to be associated with certain endocrine disorders in men in particular hypogonadism in men (Burtis etal., 2008).

In the present study 50male with type 2 diabetes as case group and 50 male without type 2 diabetes as control group were included to analyzed total testosterone level, Date was analyzed by SPSS computer program , according to results of this study; levels of total testosterone was significantly decreased in male with type 2diabetes when compared with non diabetics male, These results are in agreement with study done by onah etal ., (sep-oct,2013) in Nnewi, south eastern Nigeria in which the study showed the total testosterone was significantly decreased in male with type 2 diabetes at baseline level as compared to control group.

Our study shows that insulin sensitivity which the cause of lowered total testosterone.

And this our suggestion may be According to Dandona et al., (2009) insensitivity to insulin at the hypothalamic level may cause the development of hypogonodotrophic hypogonadism because insulin is associated with an increased concentration of inflammatory proteins in the blood which directly suppress the release of gonadotrophin -releasing hormone from the hypothalamus, or according to Pitteloud etal.,(2005) Hyperinsulinemia, as encountered in insulin resistance, might impair testosterone secretion by the Leydig cell, maybe directly since there are insulin receptors on the Leydig cell.

Our study showed a significant negative correlation between age of male diabetics' and level of total testosterone our finding supported by Nieschlag etal.,2004

Our result also showed a significant negative correlation between BMI of diabetics male and total testosterone level our finding supported by Dheeraj Kapoor etal.,2007 A plausible explanation for this is the hypogonadal –obesity- cycle, which we have recently extended (Kapoor et al.,2006). The cycle was first described by Cohen (1999).Essentially; visceral adipocytes have a high activity of the enzyme aromatase, which converts testosterone to estrogen .Testosterone inhibits the enzyme lipoprotein lipase, which takes up free fatty acids into adipocytes (Marin etal., 1995). Lower

levels of testosterone result in increased triglyceride levels in adipocytes, which promotes further a dipocyte proliferation and hence higher aromatase activity. Testosterone levels are further lowered as a result of leptin resistance at the hypothalamic pituitary LH release and testosterone secretion(Isidori etal.,1999).

Our result also showed a significant negative correlation between HbA1C of diabetics And total testosterone level our finding supported by Svartberg etal.,2004.

Our study also showed no correlation between duration of diabetes and level of total testosterone, and to the best of our knowledge no pervious study have results regarding this correlation In concluded, duration of diabetes have an insignificant role in the level of testosterone in diabetics male.

5.2. Conclusion:

The study results finding revealed that:

1-The level of total testosterone are decreased in male with type 2diabetes mellitus.

2-The level of total testosterone are negatively correlated with age, body mass index and glycated hemoglobin.

3- The level of total testosterone are no correlated with duration of diabetes.

5.3. Recommendations:

1-More studies and research must be done to explain the mechanism for total testosterone deficiency in diabetic patient.

2-Total testosterone concentration must be monitoring in diabetic patient by physician to avoid hypogonadism in diabetic patient.

3- For reliable results further study with large sample size with inclusion of fSH ,LH and free testosterone are recommended to be done.

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.

Abbendices

Appendix I

Informed consent

الموافقة المستنيرة

انا الباحثة عبير محمد ادم حمزة من كلية علوم المختبرات الطبية قسم الكيمياء السريرية ، جامعة السودان للعلوم والتكنولوجيا ، اقوم بدراسة علمية بحثية لنيل درجة الماجستير بعنوان تقييم مستوى هرمون التستيرون الكلى في مصل الدم وثاثير مرض السكرى من النوع التاني على مستوى هرمون التستيرون الكلى بين الذكور السودانيون في ولاية الخرطوم.

وفقا للدراسات سابقة فقد وجدت تناقص في مستوى هرمون التستيرون الكلى عند الذكور المصابين بمرض السكرى من النوع التاني .

لقد قمنا باختيارك لتشاركنا هذا البحث العلمي لانك تنطبق عليك كل شروط الشخص الذى يمكن ان يكون ضمن المشمولين في الدراسة.

خلال الدراسة سوف نقوم باخذ5 مل من الدم لاجراء تحليل مستوى هرمون التستيرون الكلى وايضا ساقوم بطرح بعض **الاسئلة** التي تتعلق بالعمر ومدة الزمنية لمرض السكرى واخذ الطول والوزن .

علما بان سحب العينة قد يؤدي الى احداث بعض الالم وقد يؤدي ايضا الى ظهور ورم في منطقة الحقن قد يتفشى بمرور ساعات، وظهور كدمات زرقاء وسوف نعمل على تفادي كل هذة المضاعفات.

بعد الموافقة منك واخذ المعلومات التي تتعلق بالعمرو المدة الزمنيه لمرض السكرى والاجابة على جميع الاسئلة المطلوبة في البحث ، سوف يتم اخذ العينة والعمل على تحليلها وسيتم اخبارك بالنتائج التي حصلنا عليها خلال شهرين من زمن سحب العينة ، والتي سوف تكون في سرية تامة ولن يطلع عليها احد غير العاملين في البحث بدون الاشارة الى محددات الهوية ، علما بان اشتراكك سيكون طواعية ويمكنك الانسحاب من الاشتراك في اي وقت تشاء دون اي خسائر، ونحيطكم علما انه لن تكون هناك اي عوائد مالية نتيجة اشتراكك ، ويمكنك الاتصال على الباحث في الرقم ..... في اي وقت للمزيد من المعلومات خلال فترة البحث .

اقرار المشاركة :

لقد اطلعت على المعلومات الحالية والتي تم شرحها لي واتيح لي فرصة طرح الاسئلة عنها كما شئت، ولقد تلقيت الاجابات الوافيه عن كل الاسئلة ، وانا اقر بالموافقة على المشاركة طواعية في هذة الدراسة واعلم بحقي في التوقف عن المشاركة في اي وقت دون ان يؤثر ذلك على حقوقي الاخرى او الاستفادة من نتائج هذة الدراسة.

توقيع المشارك : _____ ***ت- المشارك ----------***

توقيع الباحث: _____

بسم الله الرحمن الرحيم

Sudan University of science and technology

College of graduated studies

College of Medical laboratory science

Department of clinical chemistry

Questionnaire No

Age**……..**years

Weight**……**kilogram

Height**……..**centimeter

Duration of diseases**……**….years

Clinical investigation

BMI**……..**Kg/m²

Total testosterone**……**ng/ml

HbA1c **……%**

Signature**……..**date**…….**