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Assessment of hypogonadism and associated risk factors among male patients with type 2 Diabetes Mellitus attending Diabetic Clinic of Tikur Anbessa Specialized Teaching Hospital in Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Sisay Teka entitled " **Assessment of Hypogonadism and Associated Risk Factors Among Male patients with type 2 Diabetes Mellitus attending Diabetic Clinic of Tikur Anbessa Specialized Teaching Hospital in Addis Ababa, Ethiopia**" submitted in partial fulfillment of the requirements of the Degree of Masters of Sciences in Clinical Laboratory Sciences (Clinical chemistry) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Advisor _____Signature _____Date_____

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Abstract

Background: Global estimate of world health organization (WHO) indicated that 422 million adults aged over 18 years were living with diabetes in 2016. Type 2 diabetes Mellitus (T2DM) accounts for 90 to 95% of the incidence of diabetes. The increased prevalence of T2DM has resulted in increased prevalence of hypogonadism which is proved to be linked to it by many researchers. This in turn creates a substantial public health burden in terms of inadequate sexual function, potential infertility and poor quality of life. However, hypogonadism is under recognized and under treated in sub-Saharan African countries including Ethiopia. Therefore, the finding of this research regarding prevalence and risk factors of hypogonadism will alert clinicians and health policy makers to give attention to this problem and initiate the need for further research and appropriate intervention.

Objectives: To assess hypogonadism and its associated risk factors in men with T2DM attending Diabetic Clinic at TASTH, Addis Ababa, Ethiopia.

Method: A cross-sectional study was conducted among 115 male patients with T2DM from February to April 2017 at Diabetic Clinic of TASTH in Addis Ababa, Ethiopia. Demographic data were collected using a structured questionnaire. Clinical data were obtained from medical records. Anthropometric indices were determined. Clinical assessment of androgen deficiency was done using ADAM questionnaire. TT, LH, and FSH were determined by ECLIA method with Cobas e 411, Elecsys® 2010 analyzer. HDL-C, LDL-C, TC and TRIG were determined by enzymatic colorimetric method with Cobas 6000 module 501whereas FBG was determined by glucose oxidase method with Mindray-200E.

Results: Among the total 115 male study participants with T2DM, 104 (90.4%) had androgen deficiency symptoms but only 29(25.2%) had testosterone deficiency [TT≤12.1nmol/L]. However, hypogonadism was observed in 27(23.5%) of which 20(74.1%) and 7(25.9%) were with secondary (HH) and primary hypogonadism, respectively. Age, duration of diabetes, monthly income, alcohol consumption, and diabetic complications were not statistically associated with TT level except hypertension. BMI, WC, FBG, TRIG were negatively and significantly correlated with TT with ($r=-0.363$, $p<0.001$) ;(-0.465, $p<0.001$); ($rho=-0.328$, $p=0.001$) ;($rho=-0.357$, $p<0.001$), respectively whereas HDL-C was positively and significantly correlated with TT with ($r=0.339$, $p<0.001$). However, SBP, DBP, TC, LDL-C, LH and FSH were not significantly correlated with TT. BMI, WC, FBG, TRIG are significantly increased in hypogonadal group but HDL-C was significantly decreased in hypogonadal group with p value <0.05. WC and FBG were identified as independent risk factors for hypogonadism.

Conclusion: Though symptoms of hypogonadism are highly prevalent among men with T2DM in this tertiary care, testosterone deficiency were less prevalent. Visceral obesity and hyperglycemia are independent risk factors for hypogonadism.

Keywords: T2DM, low serum testosterone, hypogonadism, prevalence, risk factors

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ADAM	Androgen deficiency in aging men	HPG	Hypothalamic pituitary gonadal
ASA	American society of andrology	IDF	International diabetic federation
BT	Bioavailable testosterone	ISA	International society of Andrology
BMI	Body mass index	LDL-C	Low density lipoprotein cholesterol
CKD	Chronic kidney disease	LH	Luteinizing hormone
CVD	Cardiovascular disease	LST	Low serum testosterone
DBP	Diastolic blood pressure	SHBG	Sex hormone binding globulin
EAA	European association of andrology	SBP	Systolic blood pressure
ED	Erectile dysfunction	TC	Total cholesterol
ECIA	Electrochemiluminscent immunoassay	TRIG	Triglyceride
EAU	European association of urology	T2DM	Type two diabetes Mellitus
FBG	Fasting blood glucose	TASTH	Tikur Anbesa Specialized Teaching Hospital
FDA	The food and drug administration	T1DM	Type 1 diabetes Mellitus
FSH	Follicle stimulating hormone	T2DM	Type 2 diabetes mellitus
FT	Free testosterone	TNF	Tumor necrosis factor
GnRH	Gonadotropin-releasing hormone	TRT	Testosterone replacement therapy
HDL -C	High density lipoprotein cholestrol	TT	Total testosterone
ISAM	International Society for the Study of Aging Male	WC	Waist circumference
HH	Hypogonadotropic Hypogonadism	WHO	World Health Organization

Operational definitions

Low TT- TT≤12.1nmol/l

Normal TT-TT>12.1nmol

ADAM positive- If a male T2DM patient responds to question 1 and 7 or 3 other questions.

Hypogonadism - ADAM positive and TT≤12.1nmol/L

Hypogonadal: Male T2DM patient with hypogonadism

Eugonadal: Male T2DM patient without hypogonadism

Secondary hypogonadism (hypogonadotrophic hypogonadism) - Hypogonadism with either low or normal FSH (≤14 mIU/ml), LH (≤7.8 mIU/ml) or both

Primary hypogonadism: hypogonadism with elevated serum FSH (>14 mIU/ml), LH (>7.8 mIU/ml) or both

Underweight – Male T2DM patient with BMI of less than 18.5kg/m^2

Overweight- Male T2DM patient with BMI between 25 and 29.9 kg/m^2 .

Obese: Male T2DM patient with BMI of 30 kg/m^2 and above.

Centrally obese – Male T2DM patient with WC ≥94 cm.

Hypercholesterolemia -TC ≥200 mg/dl.

Low HDL - HDL-C <40 mg/dl.

High LDL -C ≥100 mg/dl.

Hypertriglyceridemia - TRIG ≥150 mg/dl.

Dyslipidemia - one or more abnormalities in serum lipids

1. Introduction

1.1. Background

Hypogonadism was defined solely on the basis of testosterone levels, but recently it is defined as clinical condition consisting of both symptoms and biochemical signs of testosterone deficiency [1]. Testosterone is the main androgen hormone which has several biological roles which include stimulating the development of male secondary sexual characteristics during puberty and their maintenance thereafter, promoting haemoglobin synthesis and red blood cell production; helping the maintenance and development of sexual function in men; stimulating anabolic muscular development and bone growth; suppressing adipose tissue formation, stimulating the basal metabolic rate which has effects on mood and cognitive ability and mediating sexual behavior and competitive encounters [2].

In eugonadal men testosterone production is regulated by the hypothalamic–pituitary–testicular (HPG) axis [**figure 1**]. The hypothalamus secretes gonadotropin-releasing hormone (GnRH) that stimulates the anterior pituitary gland to produce follicle stimulating hormone (FSH) and luteinizing hormone (LH). LH acts on the interstitial Leydig cells of the testes, stimulating them to produce testosterone, whereas FSH stimulates spermatogenesis and Sertoli cell function. The HPG axis is regulated by a negative feedback mechanism. Testosterone inhibits the frequency and amplitude of GnRH release from the hypothalamus and also the secretion of LH from the pituitary. The Sertoli cells of the testes, in addition to stimulating spermatogenesis, also secrete the glycoprotein hormone inhibin, which provides negative feedback to the pituitary, inhibiting the secretion of FSH [3].

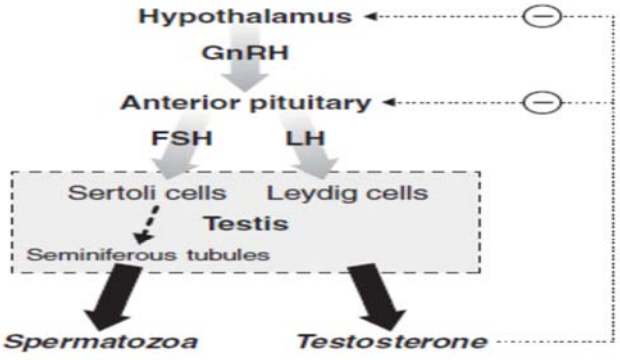

Figure 1: The hypothalamic–pituitary–gonadal axis in men [Obtained from: Dandona P et al,2010]

Testosterone exists in different forms in blood circulation. Only 1–2% of testosterone circulates free in the blood; the remaining 98–99% is bound to albumin (40–50%) and to sex hormone binding globulin (SHBG) (50–60%). Testosterone binds strongly to SHBG, and it is therefore largely the free and albumin-bound testosterone that is available for biological action. For this reason, free testosterone (FT) and albumin-bound testosterones together are termed bioavailable testosterone (BT) [4].

The association between low serum testosterone and type 2 Diabetes Mellitus (T2DM) has recently drawn substantial attention [5]. In men with T2DM about 25–40% has low testosterone concentrations in association with inappropriately low or normal LH and FSH concentrations and diagnosis of hypogonadotrophic hypogonadism (HH) can be established. However, about 4% of men with T2DM have subnormal testosterone concentrations with elevated LH and FSH concentrations, which can be associated with primary testis dysfunctions [6].

The exact mechanisms underlying the occurrence of hypogonadism in men with T2DM remain unclear yet. However, there are suggested pathophysiological mechanisms as depicted in **(figure 2).**The first possible mechanism is that an excessive increase in fat mass may result in an increase in the activity of aromatase enzyme, which causes greater conversion of testosterone into estradiol. This estradiol directly feeds back and inhibits the HPG axis through kisspeptin [7]. Second, increase in adipocytokines due to obesity, including the pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin-1β, and interleukin-6 inhibit the secretion of testosterone, both at the hypothalamic–pituitary and the testicular level [8]. Third, leptin usually stimulates the release of GnRH; however, in obesity, where excess leptin is produced from adipocytes, the hypothalamic–pituitary axis becomes resistant to leptin [9]. In addition, leptin inhibits the stimulatory action of gonadotropin on the Leydig cells of testes, thereby further decreasing testosterone production [10]. Fourth, it is becoming increasingly evident that the action of insulin and responsiveness to insulin in the brain are necessary for the adequate function of the hypothalamo-hypophyseal axis (hypothalamus, pituitary gland, testes). Thus, insensitivity to insulin at the hypothalamic level may contribute to the development of HH. Insensitivity to insulin is also associated with an increased concentration of inflammatory proteins in the blood. These proteins may also directly suppress the release of GnRH from the hypothalamus [11].

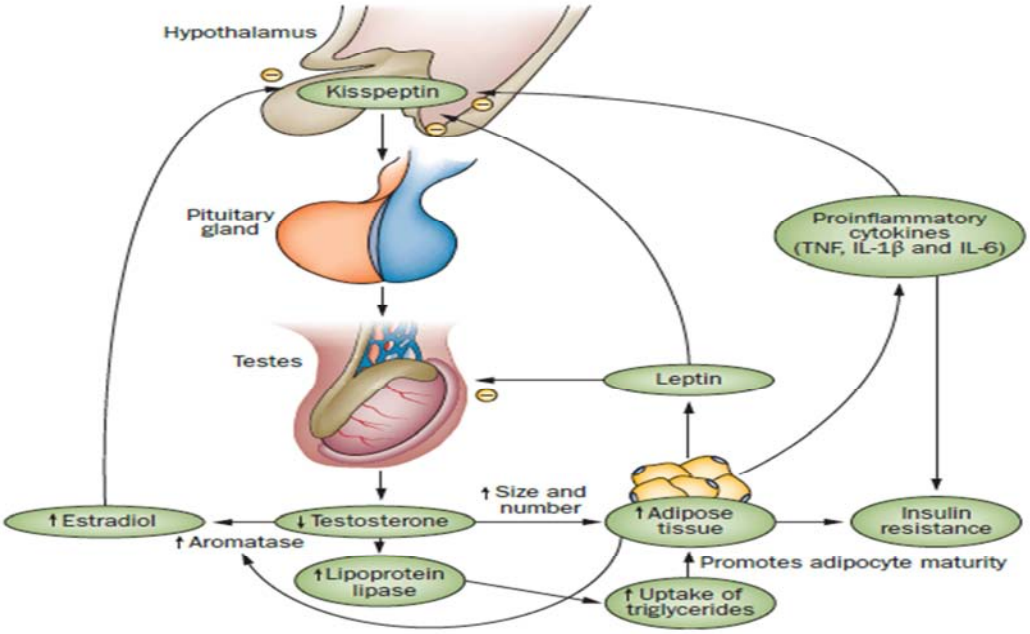

Figure 2: The hypogonadal–obesity–adipocytokine hypothesis [obtained from PM et al, 2013]

Adipokines causes vascular endothelial dysfunction, which are potential causative factors for increased cardiovascular disease and erectile dysfunction(ED). The low testosterone level in men with T2DM is then associated with diminished libido, ED, increased fat mass, decreased muscle mass, decreased bone mass and energy, depression, and anemia [5]. There is also a growing evidence that hypogonadism is a risk factor for coronary artery disease, the leading cause of mortality in patients with T2DM [12]. Other adverse effects reportedly associated with hypogonadism include poor quality of life, increased fracture risk; cognitive decline, and mortality [13]. Additionally, total testosterone (TT) level can predict development of the hypertension, dyslipidemia and hyperglycemia in middle-aged men with T2DM [14].

A study identified several risk factors correlate of T2DM associated closely with hypogonadism such as age, visceral obesity, hyperglycemia, dyslipidemia, and hypertension in male patients with T2DM [15]. Another study also showed that hypertension, anthropometric indices (BMI and WC), advanced age, current smoking, and general health status are considered as the risk factors of hypogonadism [16]. Therefore, T2DM and hypogonadism have bidirectional relationship.

1.2.Statement of the problem

In 2015, globally 415 million adults had diabetes. This number is expected to exceed 642 million by 2040. By 2040 this will rise to 642 million. In Africa more than 14 million people had diabetes. This figure will reach 34 million by 2040. Africa has the highest percentage (66.7%) of undiagnosed people, who are at higher risk of developing harmful and costly complications. Diabetes caused 321,000 deaths in 2015.Over 1.33 million estimated cases of diabetes in Ethiopia with prevalence estimate of 2.9% in which 23,145 diabetes-related deaths were also reported in 2015 [17]. T2DM accounts for 90 to 95% of the incidence of diabetes [18].

A review by Dandona P etal in 2009 summarized that there is high prevalence of hypogonadism in men with T2DM worldwide [19]. A Southern California population-based cohort of 985 men aged 40–79 years published in 1990, indicated that 110 men with diabetes had significantly lower mean levels of endogenous plasma testosterone than did 875 non diabetic men [20]. In 2004, a cross-sectional study conducted in Newyork had also revealed association between T2DM and lower TT levels [21]. Hackett G in his review 2010 summarized there is significant association between testosterone and T2DM [22]. Additionally, a cross sectional study in New york on young T2DM patients also revealed that significantly lower plasma concentrations of TT and FT and inappropriately low LH and FSH concentrations with a very high prevalence of HH among men with T2DM [23].

High prevalence of symptomatic hypogonadism creates a substantial public health burden in terms of inadequate sexual function and potential infertility. The problem of HH which is the most prevalent in T2DM patients is not confined only to sexual and reproductive function but also associated with visceral obesity, hyperglycemia, dyslipidemia, hypertension, cardiovascular disease, and ED [19].

Improving sexual health is a portal to identify health hazards and improving men’s health. Appropriate diagnosis and medical work up of men presenting with sexual symptoms may have the benefit of the diagnosing and treating other important conditions, such as obesity, diabetes, hypertension and hyperlipidaemia. However sexual health problems including hypogonadism are under treated [24].

An Endocrine Society task force in 2010 recognized the link between hypogonadism and T2DM and recommended physicians to consider measuring testosterone in all men with T2DM [1]. An International society of Aging Male also recommended measurement of testosterone in men with T2 DM in 2015 [4]. The paradox is that testosterone levels are not routinely measured according to this recommendation, meaning that physicians do not put themselves in a position to detect hypogonadism and make appropriate clinical decisions [22]. Additionally, sexual dysfunction associated with hypogonadism in men with T2DM is often undiagnosed and under-discussed unless the healthcare provider specifically asks the patient about possible symptoms. Because this is a conversation that many patients make personal and feel uncomfortable about initiating it due to taboo or stigma [26]. Another realistic explanation is that the target-based management of T2DM is already created a workload for clinicians and that routine questioning about ED and other symptoms of hypogonadism and associated testosterone estimation will lead to considerable additional workload, without resources or remuneration [22].Moreover, in a resource limited country like Ethiopia it is difficult to implement routine measurement of testosterone for all T2DM patients.

In spite of the limited resource, it can be considered at least for symptomatic hypogonadal men with T2DM after screening them with appropriate standard questioner or clinical assessment to identify patients who can benefit from testosterone replacement therapy. Particularly, testosterone level of T2DM patients presented with ED should be checked. This is because, these patients with low testosterone do not usually respond to treatment of phosphodiestrase inhibitors like sildenafil citrate. Therefore, failure to measure testosterone in these patients results in considerable waste of resources and extend the time to find alternative treatment for the patients unnecessarily [22,25].

Additionally hypogonadism and its associated risk factors are largely under studied in sub-Saharan Africa despite the continent having a high burden of T2DM. Ethiopia is no exception. Therefore assessing prevalence of hypogonadism and which risk factors correlate of T2DM is associated with it is crucial in Ethiopian context.

1.3. Significance of the study

Few studies in Sub-Saharan Africa have been done and published regarding prevalence of hypogonadism and its associated risk factors among men with T2DM, but as to our knowledge none in Ethiopia. The data generated from this research showed the prevalence of symptomatic hypogonadism and its associated risk factors in men with T2DM attending Diabetic Clinic of TASTH. Therefore, determining prevalence and risk factors of hypogonadism will alert clinicians and health policy makers to give attention to this problem and initiate the need for further research, appropriate intervention and its inclusion as part of routine diagnosis in symptomatic men with T2DM.

Testosterone is not measured routinely in this Hospital in spite of Endocrine society guideline suggesting that it should be always be measured in men with T2DM specially those who present with symptoms. Measuring TT among T2DM patient with symptoms of hypogonadism especially ED is important to identify patients who can benefit from testosterone replacement therapy. Additionally, it was established that men with ED who fail to respond to phosphodiesterase type 5 inhibitors are more likely to have low serum testosterone and therapeutic potential of phosphodiestrase inhibitors will only become manifested in eugonadal men with ED. As a result, provision of this treatment before determining testosterone level of the patients is a waste of resources. Therefore, the finding will also help to indicate the need for routine measurement of TT, LH and FSH in symptomatic hypogonadal male with T2DM specially for those with ED .

This study identified major risk factors for hypogonadism related to T2DM. Identifying those risk factors may help in prevention, early diagnosis, and early treatment of hypogonadism. Because studies showed that some of the risk factors for hypogonadism related to T2DM like hypertension, dyslipidemia, BMI and WC are modifiable to decrease the rate of development of hypogonadism and its symptoms. Furthermore, the finding of this study will also be the basis for future research among this population with large sample size.

2. Literature review

2.1. Association of low serum testosterone and type 2 diabetes Mellitus

According to WHO global estimation, 422 million adults aged over 18 years were living with diabetes in 2016 [27]. T2DM accounts for 90 to 95% of the incidence of diabetes and is associated with a strong genetic predisposition as well as age, obesity and lack of physical activity [19]. In 2006, a systematic review and meta-analysis by Ding EL and his coworkers confirmed that men with type 2 DM have significantly lower levels of testosterone than non-diabetic controls [28]. As summarized in a review made by Dandona P and colleagues in 2011, the most common hypogonadism in men with type 2 DM is secondary hypogonadism or HH [5].

The link between hypogonadism and T2DM was also shown in a case control study conducted by Verma S etal in 2013 in India and a cross-sectional study conducted in Nigeria in 2011 by Ogbera OA , respectively [29,30]. In 2006, a cross sectional by Corona G etal identified that the mean total testosterone levels and free testosterone levels were significantly lower in T2DM patients than in the rest of the sample and this difference was retained statistically significant after adjustment for age [31]. Chandel A etal conducted a similar study in 2008 among 38 Type 1 Diabetes Mellitus (T1DM) and 24 T2DM young patients aged ranged 18–35 years in Western New york, concluded that T2DM patients had significantly lower plasma concentrations of TT and FT. This group of patients had also inappropriately low LH and FSH concentrations with a very high prevalence of HH, when compared with type 1 diabetic patients of a comparable age [23].

Farrell JBA and colleagues in their meta-analysis of 26 studies published in 2008 summarized that there is a link between low levels of testosterone and an adverse metabolic profile (ie, obesity and insulin resistance). In the analysis they indicated that hypogonadism is associated with metabolic syndrome and T2DM in men in all the studies [32].A review by Wang C et al which was published in 2011 noted that low TT or SHBG levels are associated with T2DM, independent of age, race, obesity, and criteria for diagnosis of diabetes [33].

Goto A etal and his team selected 300 diabetic cases and 300 non diabetic controls from Saku cohort conducted between 2009-2010 in Japan and conducted a cross sectional study. The study showed that FT had strong positive correlation with TT and a strong inverse correlation with SHBG in both men and women [34].

Another similar study by Rabijewski M etal published in the same year in a 184 T2DM and 149 non diabetic control men in Poland showed that TT and cfT concentrations in all T2DM group were statistically significantly lower than in non-diabetic control group with the same average age [35]. Almihya NF et al conducted a similar study in Egypt which aimed to assess the link between T2DM and the increased incidence of hypogonadism in men with T2DM, indicated that the patient group showed a significant decrease in serum TT and FT in comparison with the control group in 2015[11] .

Cross sectional studies also showed that there is a significant positive correlation of low TT with FSH and LH in men with T2DM. A similar study of 103 T2DM patients which was conducted in Newyork by Dhindsa S and colleagues identified that FSH and LH levels were significantly lower in the hypogonadal group compared with patients with normal FT and TT levels [11, 21].

2.2. Association of low serum testosterone with age, obesity, hypertension and dyslipidemia

It is well established that age has significant effect on both TT and FT levels, with the levels decreasing with advancing age. In Baltimore Longitudinal study on aging, TT levels dropped to hypogonadal levels in about 20% of men older than 60 years, 30% over the age of 70 years, and 50% in men above 80 years of age. This is due to decrease in testicular response to gonadotropins [36]. A cross sectional study conducted in England by Kapoor D et al in 2007 also showed negative correlation between age and both TT and FT levels [25]. However, age did not show correlation with TT in a study conducted in New York [21].

Dandona PM and Rosenberg TA in their review published in 2010 summarized that low serum testosterone is also associated with obesity. Obesity in turn, strongly associated with T2DM: approximately 83% of T2DM are overweight or obese [2]. There is an inverse linear

relationship between TT and BMI, the indicator of obesity [15, 21, 23 25, 29, 31] and there is also inverse relationship between TT and WC, an indicator of visceral obesity [15, 25, 29].

In a cross sectional study conducted in Italy by Crona G etal showed that men with T2DM showed lower HDL and higher triglyceride when compared to the rest of the sample. These differences were confirmed after adjustment for age and/or BMI [31].However, a study conducted in Poland to assess risk factors of hypogonadism reported that there were no correlations between TT and HDL-C or TT and TRIG [15].

2.3. Prevalence hypogonadism among men with type 2 diabetes

A cross-sectional study of 1,292 men by Brand et al reported that men with T2DM had not only lower testosterone but also lower levels of SHBG when compared with non-diabetic men [37]. In a similar study of 1246 Italian men in 2008 by Farrell JB etal , 24.5% men with T2DM versus 12.6% prevalence of hypogonadism in the rest of the sample with TT cutoff value less than 10.4nmol/L. This differences in the prevalence of hypogonadism retained significance after adjustment for age and BMI [32]. Another similar study conducted in Isfahan, Iran by Mirzaei MR and colleagues on 247 men with T2DM reported 18(7.4%) prevalence of overt hypogonadism and 24 men (9.9%) with borderline hypogonadism in 2011 [38]. In 2014, Hayek AA et in similar study in Jordan among 1717 men (1089 participants with type 2 diabetes and 628 non-diabetic subjects) reported that the prevalence of hypogonadism among all study participants was 18.5%. The prevalence of hypogonadism in diabetic and non-diabetic men was 24.3% and 8.3%, respectively. In response to (ADAM) questionnaire, 19.8% of diabetics and 3% of the non-diabetics had symptomatic androgen deficiency [39].

In 2016, Ugwu TE. et al conducted cross-sectional survey of T2DM males aged 32–69 years in Nigeria indicated that overt and possible hypogonadism occurred in 29.5% and 23% of the participants, respectively. Majority (76.3%) of the subjects who had overt hypogonadism had the HH pattern [40]. A similar study of 50 diabetics of 30-76 years age group in Ajimir , India, by Khan I and colleague, determined a prevalence of 30% of overt hypogonadism

(TT <8 nmol/l) and 28% prevalence of borderline hypogonadism(8-12 nmol/l) in men with T2DM [41].

Asare-Anane H et al conducted a case control study in Ghana involving a total of 105 T2DM male and 105 healthy controls between 30 and 60 years .The researchers reported that incidence of hypogonadism was five times more in the diabetic subjects than non-diabetic controls. A total of 37 (35.2%) of the diabetic men and 7 (6.7%) of the control subjects were hypogonadal. Also, 21 (20%) and 10 (9.5%) had testosterone levels between borderline group for the diabetic men and non-diabetic men respectively [42].

In 2015, Kempa T and Reader P in their publication of an observational cross sectional study of 150 consecutive male patients aged 50 years and older in South Africa, the testosterone deficiency symptoms was found in 94.7% the participants. However, some 50% of the men had low total testosterone levels [43]. Another cross sectional study conducted in 300 Sudanese men with T2DM and 100 controls without T2DM by Mohieldin JM et al and indicated 234(78%) 234 men with T2DM with TT cutoff value of less than10.4 nmol/L,. In the control group only 10(10%) were hypogonadal [44].

3. Objectives of the study

3.1. General objective

To assess hypogonadism and its associated risk factors in men with T2DM attending diabetic clinic of Tikur Ambessa Specialized Teaching Hospital in Addis Ababa, Ethiopia.

3.2. Specific objectives

- To determine the prevalence of hypogonadism using total testosterone and clinical symptoms among men with T2DM.
- To evaluate the test characteristics of ADAM questioner in identifying male patients with T2DM having low total testosterone.
- To determine the association between demographic, clinical and biochemical parameters with serum total testosterone
- To determine which demographic, clinical, and biochemical variables significantly differ between hypogonadal and Eugonadal group.
- To identify potential risks factors associated to primary and secondary hypogonadism among men with T2DM.

4. Hypothesis

- ✓ The prevalence of hypogonadism in male T2DM patients attending follow up treatment at TASTH is higher than those reported in the literature.
- ✓ There is no significant difference in and biochemical variables between hypogonadal and Eugonadal group of T2DM patients attending follow up treatment at TASTH.

5. Material and methods

5.1. Study area

Tikur Anbesa specialized hospital is located in the capital Addis Ababa, Ethiopia. It is Ethiopia's largest specialized public tertiary referral Hospital and one of University Hospitals in the country where patients from all over the country get referral service. The Hospital provides a tertiary level referral treatment and is open for 24 hours for emergency services. It offers diagnosis and treatment for approximately 370,000- 400,000 patients a year. There are different units and clinics that provide specialized service for clients. Among these clinics the diabetes clinic where we selected our study participants was inaugurated in1994 by Lion's club. The clinic is serving diabetic patients coming from different regions of the country [45].

5.2. Study design and period

A cross-sectional study was conducted from February to April, 2017.

5.3. Population

5.3.1. Source population

All male type 2 diabetes out-patients on follow up treatment in diabetes Clinic of TASTH, Addis Ababa Ethiopia.

5.3.2. Study population

All male type 2 diabetes out-patients who have treatment follow up appointment during data collection period at Diabetic Clinic of TASTH, Addis Ababa Ethiopia.

5.4. Inclusion and exclusion criteria

5.4.1. Inclusion criteria

- Male patients diagnosed and confirmed to be with type 2 diabetes and attended treatment follow up for at least one year were included in the study
- Patients whose age was above 35 years were included.
- Patients who were capable of independent communication and giving informed consent were considered to be the participants of the study

5.4.2. Exclusion criteria

- Patients on testosterone replacement therapy
- Patients who are taking glucocorticoids
- Patients with known history of chronic diseases like liver cirrhosis, cancer, and AIDS were excluded

5.5. Study variables

5.5.1. Dependent variables

Hypogonadism

5.5.2. Independent variables

- Age
- Duration of diabetes
- Anthropometric indices :Waist circumference and body mass index (WC, BMI)
- Diabetic complications: retinopathy, neuropathy and nephropathy
- History of hypertension
- History of smoking
- Alcohol consumption
- Serum LH level
- Serum FSH level
- Fasting blood glucose concentration
- Serum lipids :HDL-C,TRIG, LDL-C and TC

5.6. Measurement and data collection procedure

5.6.1. Sample size determination

The actual sample size for the study was determined using the formula for single population proportion by assuming 5% marginal error (d), 95% confidence interval (α=0.05). The value of p taken was 7.4% from previous study conducted in Iran in 2012 on hypogonadism in diabetic men [38]. Based on the above information the total initial sample size was calculated by using the following formula.

$$n_i = \frac{(Z\ \alpha/_2)2\ pq}{d^2}$$

Where; n_i= required initial sample size

$Z_{\alpha/2}$ =critical value for normal distribution at 95% confidence interval which equals to 1.96 (Z value at alpha=0.05).

P= the prevalence of hypogonadism in Iran using TT in T2DM patients = 7.4%

q= Proportion of Sudanese type 2 diabetic population not having hypogonadism=92.6%

d= marginal error =0.05

n $= (1.96)^2\ (0\ .074 \times 0.926)\ /[(0.05)^2] = 105$

Taking 10% contingency a total sample size was calculated to be 121.

5.6.2. Sampling method

Convenient sampling method was used to select 121 study participants

5.6.3. Demographic and clinical data collection procedure

Their demographic characteristics, smoking habits, alcohol consumption, and history of hypertension were collected using a structured questionnaire (**Refer Annex III**). Duration of diabetes, presence of retinopathy, neuropathy, nephropathy, hypertension and current medication were collected from the medical records by using checklists (**Refer Annex IV**). Anthropometric characteristics of study participants: height and weight. BMI was calculated as weight (kg) divided by height squared (m^2). WC was measured at a point midway between the inferior border of the costal margin and the iliac crest in mid-axillary line to the nearest

0.5 cm. Collection of these medical records and clinical measurements were done by professional nurse to minimize bias [46].

BMI of less than 18.5kg/m^2 was considered underweight, BMI between 25 and 29.9 kg/m^2 was considered overweight, whereas an adult who has a BMI of 30 kg/m^2 or higher was considered obese. Central obesity was assessed by WC and subjects with WC ≥94 cm were regarded as obese, according to the international guideline [46].

Clinical assessment of androgen deficiency symptoms was carried out using the androgen deficiency in aging male (ADAM) questionnaire (**Refer Annex III part III**). ADAM questioner is the most widely accepted screening questionnaire which consists of 10 questions that evaluate the kind and severity of low testosterone symptoms. It has variable low specificity but it has reasonable sensitivity in the presence of low testosterone levels. A study participant was considered ADAM positive if he answered "yes" to questions 1 and 7 or to any other 3 questions or ADAM negative if he did not [47]. The questioner was translated into Amharic to make it easily to understand being assisted by a physician.

Hypogonadism was defined the presence of symptoms of testosterone deficiency and a subnormal testosterone level less than 12.1nmol/L [4]. Subjects who had hypogonadism with either low or normal FSH (≤14 mIU/ml), LH (≤7.8 mIU/ml) or both were diagnosed as hypogonadotrophic hypogonadism (secondary hypogonadism) while those with hypogonadism and elevated serum FSH (>14 mIU/ml), LH (>7.8 mIU/ml) or both were diagnosed as having primary hypogonadism [1].

Hypercholesterolemia was referred to a total cholesterol level ≥200 mg/dl. HDL was considered low when the level was <40 mg/dl. LDL was considered high when the level was ≥100 mg/dl. Hypertriglyceridemia was considered high when TG level was ≥150 mg/dl. Dyslipidemia was considered present when one or more of the previous abnormalities were found in serum lipids [46].

5.6.4. Blood sample collection, processing and laboratory analysis

Overnight fasting whole blood sample was collected from study participants using serum separator tube (BD™), before 10 am in the morning and allowed to clot for 30 minute and serum was separated by centrifugation at 1500 rpm. Serum samples were aliquated for biochemical test and immunoassay (**Refer Annex V**) .The serum samples were stored at -

20^0C until analysis in Medical Laboratory of TASTH. After completion of sample collection the stored serum samples were transported to Ethiopian public Health clinical chemistry referral laboratory for analysis.

Hormones (TT, FSH and LH) were analyzed with fully automated Analyzer called Elecsys® 2010 analyzer, cobas e 411 with ECLIA method .To measure TT competitive immunoassay principle with analyte liberation was applied (**Refer Annex V-section B**). To measure both LH and FSH sandwich immunoassay principle was used (**Refer Annex V section C and D** respectively).

Lipid profiles (LDH-C, TG, HDL-C and TC) were also analyzed in this laboratory with Cobas 6000 501 module .Enzymatic method was used to determine the level of each lipid in the serum samples [**Refer Annex V section F (i-iv)**]. Serum glucose was determined by the glucose oxidase method (**Refer Annex V section E**) with Mindray-200E, a fully automated analyzer at TASH. Glucose results were accessed on the same day of sample collection laboratory request of results using patients' Hospital card number with permission.

5.7. Data quality assurance

Pre-analytical: Blood sample quality was ensured during collection and processing by following the standard operating procedures. Samples were stored in appropriate refrigerator temperature (-20^oC) until analysis in TASH laboratory. Samples with incomplete information were rejected.

An ice box was used when they were transported to Ethiopian public health clinical chemistry Referral laboratory.

Analytical: the performance of automated clinical chemistry analyzers was checked by running quality controls.

Post analytical: results were printed out after checking appropriateness of all the test results and the data were carefully entered into Microsoft Excel worksheet and saved for statistical analysis.

5.8. Data processing, presentation and analysis

Data which were obtained from medical records, using questioners and findings from laboratory analysis were entered into Microsoft Excel and exported to SPSS version 20 for analysis. Before data analysis was carried out all continuous variables were assessed whether they are normality distributed or not visually using histogram. Data were also explored and tested by Kolmogorov-Smirnov (K-S) and Shapiro-Wilk test of normality. If $p \geq 0.05$, the data were considered as normally distributed.

Descriptive statistics were used to summarize Qualitative variables were reported with number and percentages. Graphs, tables and charts were used to present results. All parametric values were expressed as the mean ± SD whereas for non-parametric values median was reported with interquartile range. Chi-square ($\chi 2$) test was also used to compare qualitative variables. Pearson's and Spearman's correlation for parametric and non-parametric data were used to establish correlations respectively. Mann Whitney U test was used for comparing nonparametric data, and independent sample **t-**test was used to compare parametric data. One way Analysis of Variance (ANOVA) was used to compare parametric data and Kruskal -Wallis test was used to compare non parametric data with multiple categories. Multiple linear regressions to identify potential predictors of hypogonadism and multiple logistic regressions were used to identify independent risk factors for hypogonadism. P value < 0.05 was considered statistically significant

5.9. Ethical considerations

Ethical clearance was obtained from the Departmental Research and Ethics Review Committee of the Department of Medical Laboratory Sciences Allied School of Medical Laboratory Sciences College of Health Sciences of Addis Ababa University on January 1, 2017. After summiting this ethical clearance and support letter to the Department of Internal Medicine of Addis Ababa University College of health sciences, the departmental further evaluated the proposal of this research and allowed the start of the research by a letter written on March 1, 2017 to Endocrinology Unit. Written consent was obtained from each study subjects before collection of blood samples and other relevant clinical information. Security and confidentiality of detail clinical and laboratory finding data were kept carefully.

6. Results of the study

6.1. Socio-demographic and some clinical characteristics of study participants

A total of 121 male T2DM patients were recruited in this study .However, questioner or clinical information of five of them were incomplete. 116 serum samples of the participants were analyzed .However, TT of one study participant were below the detection limit and this was excluded from statistical analysis. Thus, only 115 of them were included in statistical analysis and results are presented as follows.

As summarized in (**table 1**), of 115 patients participated in our study, 44(38.3%) were in the age group of 60-69. Though not indicated in this table, the minimum and the maximum age was 40 and 80 year respectively. The median age was 60 years with 2.5^{th} and 97.5^{th} interquartile range (IQR) of 40-78.20 years.

Majority of these participants 111(96.5%) were married. 3(2.6%) of them were divorced and only 1(0.1%) was widowed. When they were categorized into their education level 55(47.8%), 36 (31.3%), and 15(13.0%) completed university or college education, secondary school, and elementary school respectively. But 9(7.8%) of them were uneducated.

Of 115 study participants, 42(36.5%) were retired. 28(24.5%) were government employees whereas, 8(7%) were NGO employees. were government employees. However, 10(8.7%) of them were unemployed. Of the remaining 25 (21.7%) subjects, 22(19.1%) were owner of private business and 5(4.3%) were farmers, respectively. With regards to their average approximate monthly income, 69(60%) were earning less than 2000 EBr. The rest 46(40%) were earning 2000 EBr and above.

Table 1: Socio-demographic characteristics of male with T2DM attending treatment at Diabetic clinic of TASTH from February to March 2017 (n=115)

Variables	Categories	Frequency	Percent
Age (Years)	40-49	18	15.7
	50-59	31	27.0
	60-69	44	38.3
	>69	22	19.1
Marital status	Married	111	96.5
	Divorced	3	2.6
	Widowed	1	0.9
Level of Education	College/University	55	47.8
	Elementary school	15	13.0
	Secondary school	36	31.3
	Uneducated	9	7.8
Employment status	Government employee	28	24.3
	NGO employee	8	7.0
	Private business	22	19.1
	Retired	42	36.5
	Farmer	5	4.3
	Unemployed	10	8.7
Monthly income(EBr)	<2000	69	60.0
	≥2000	46	40.0

As indicated in (**table 2**), of all 115 study participants only 5(4.3%) were current smokers of cigarettes whereas 26(22.6%) were past smokers. However, 84(73%) of them reported that they were never smoked. They were also asked about their history of alcohol consumption and accordingly, 47(40.9%) reported that they were drinking alcohol currently. But, 29(25.2%) reported that they stopped drinking alcohol and the rest 39(33.9%) were reported that they never drunk.

Majority of the participants 72(62.7%) were with hypertension and all of them were taking antihypertensive drugs and 91(79%) were with dyslipidemia. With regards to prevalence of diabetic complication among them, 17(14.8%), 26(22.6%) and 22(19.1%) were with retinopathy, neuropathy and nephropathy respectively. Their diabetic medications were also accessed from their medical records and summarized: 64(55.7%) were on oral hypoglycemic

agents, 28(24.3%) were on insulin and oral hypoglycemic agents. The rest 23(20.0%) were on insulin therapy alone.

The diabetes duration of the majority of men with T2DM participated in this study was less than 15 years. The median duration of was 7 with a range of 1-30 years.

Table 2 : Clinical characteristics of male with T2DM attending treatment at Diabetic clinic of TASTH from February to March 2017. (n=115)

Variables	Categories	Frequency	Percent
Smoking history	Current	5	4.3
	Past	26	22.6
	Never	84	73.0
Alcohol consumption	Current	47	40.9
	Past	29	25.2
	Never	39	33.9
Hypertension and antihypertensive drugs	Yes	72	62.6
	No	43	37.4
Retinopathy	Yes	17	14.8
	No	98	85.2
Neuropathy	Yes	26	22.6
	No	89	77.4
Nephropathy	Yes	22	19.1
	No	93	80.9
DM treatment modality	Oral hypoglycemic drugs	64	55.7
	Insulin therapy alone	28	24.3
	Insulin and oral hypoglycemic drugs	24	20.0
Duration of DM	<5	42	36.5
	5-15	37	32.2
	11-15	18	15.7
	>15	18	15.0
Dyslipidemia	Yes	91	79
	No	24	20.9

6.2. Distribution of clinical and laboratory data

Normality distributions of all continuous variables considered in our study specifically, age, WC, BMI, SBP, DBP, TT, LH, FSH, FBS, HDL-C, LDL-C, TC and TRIG were checked. Among these variables age, SBP, DBP, FBS and TRIG were failed to be normally distributed even after they were logarithmically transformed. However, BMI, WC, TT, HDL-C, and LDL-C were found to be approximately normally distributed without being transformed into $\log_{10}$ whereas TC, LH and FSH became normally distributed after they were logarithmically transformed .Only normality test result of approximately normally distributed parameters are depicted in (**table 3**).(**Refer figure 3 and 4 for normality distribution**)

Table 3: Results of test of normality, skewedness and kurtosis of some clinical and laboratory data of study participants.

	Tests of Normality				
No	**Parameters**	**Kolmogorov -Smirnov[a] (K-S)**	**Shapiro-Wilk**	**Skewedness**	**Kurtosis**
		P value	**P value**		
1	TT(nmol/L)	0.200*	0.052	0.250	-0.613
2	LDLC-C(mg/dl)	0.200*	0.209	0.270	0.465
3	HDL-C(mg/dl)	0.200*	0.264	0.125	0.408
4	WC (cm)	0.035	0.228	0.102	-0.322
5	BMI(kg/m^2)	0.179	0.195	0.382	0.107
6	TC	0.072	.044	0.418	-0.368
7	Log 10(TC)	0.200*	0.489	–0.177	-0.202
8	LH	.000	.000	1.183	1.112
8	Log_{10}(LH)	0.200*	0.294	0.244	-0.362
9	FSH	.000	.000	1.69	2.99
10	Log_{10}(FSH)	0.200*	0.514	0.217	-0.062
	*. This is a lower bound of the true significance.				
	a. Lilliefors Significance Correction				

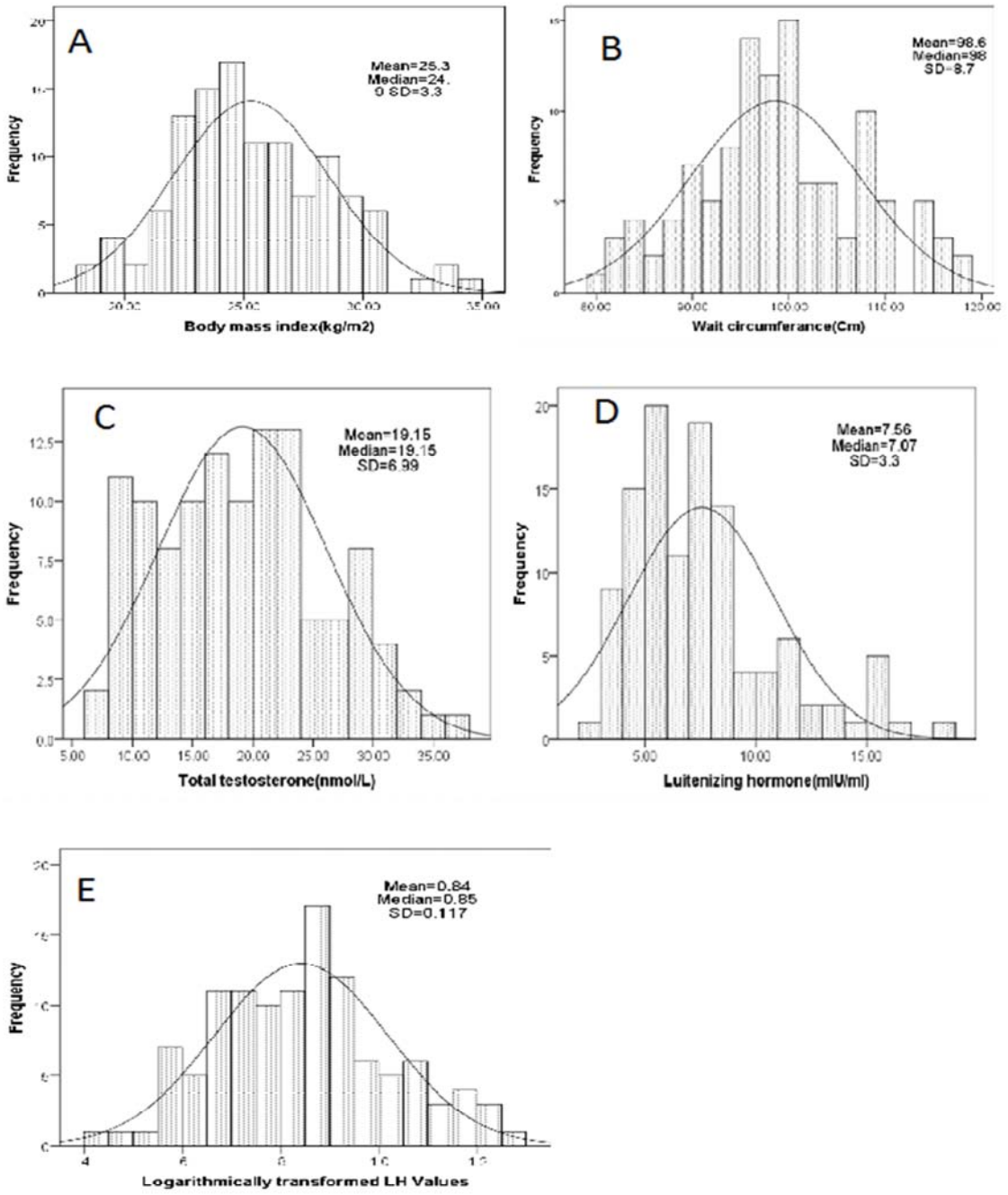

Figure 3: Shows histograms of approximately normal distribution of BMI (A), WC (B), TT(C) and logarithmically transformed LH (E).

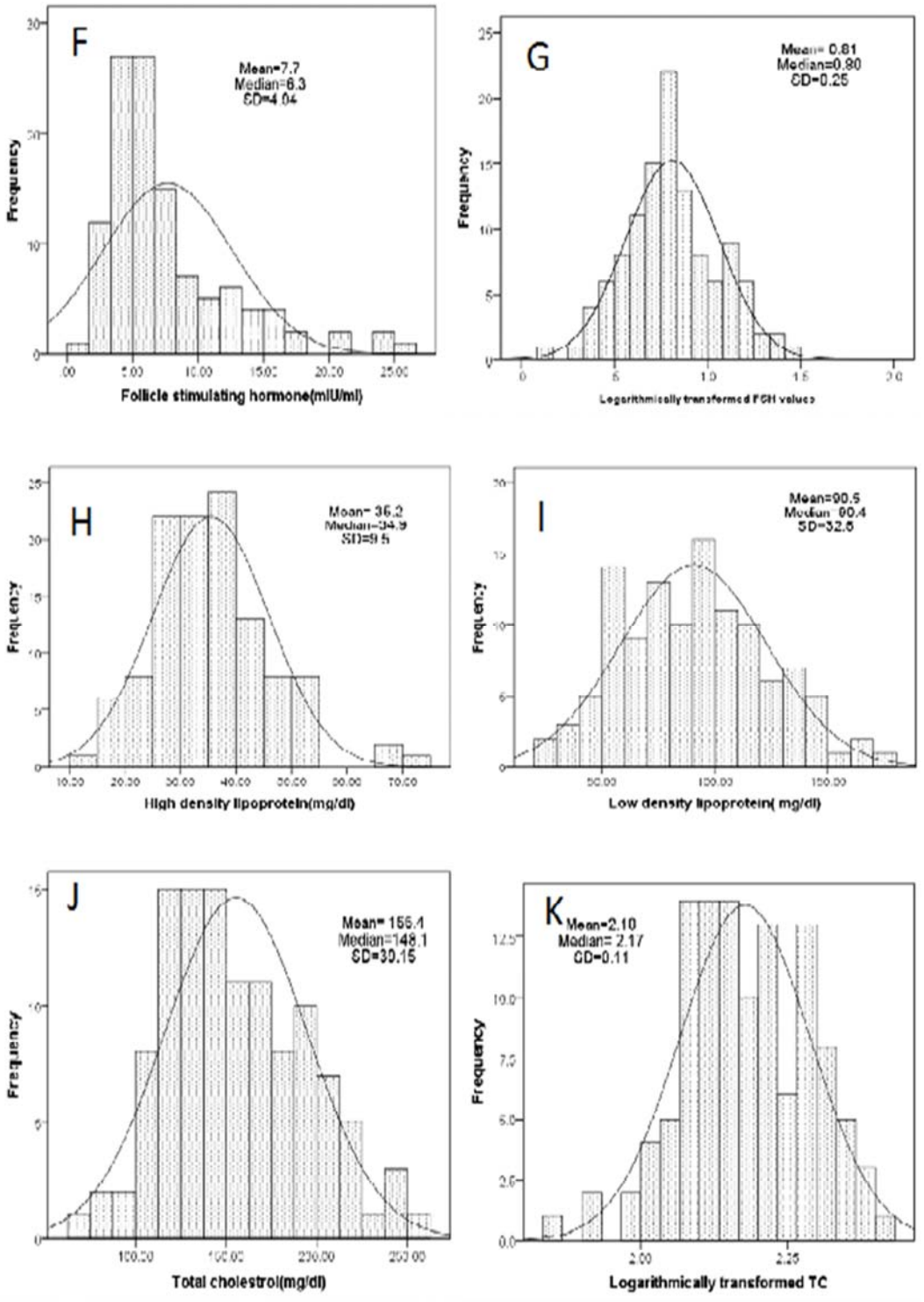

Figure 4: Shows histograms of approximately normal distribution of logarithmically transformed FSH (G), HDL-C (H), LDL-C (I), and logarithmically transformed TC.

6.3. Quality control data of hormones and lipids

Before analysis of serum samples at Ethiopian Public health Clinical Chemistry Referral laboratory, daily quality control was done on the date of analysis of the serum samples. Results are presented in **table 4**. Levey Jenning's Chart of quality control of days before our sample analysis was checked .All values were in acceptable ranges.

Table 4: Summary of Daily Quality Control result of lipid profile of Cobas 6000 c501 module and Cobas e411 on 22/04/2017 at EPHI Clinical chemistry Referral Laboratory.

No	**Quality Controls**	**Control results**			
		TC(mg/dl)	**HDL(mg/dl)**	**LDL(mg/dl)**	**TRIG(mg/dl)**
	Lipids				
1	Normal control (PCC1)	94.1	31.6	57.8	122.8
2	Pathologic control (PCC2)	167.9	60	98.1	200.3
	Hormones	**TT(mI/ml)**	**LH(mIU//ml)**	**FSH(mIU/ml)**	
1	U1(Universal -1)	5.73	10.19	15.10	
2	U2(Universal -1)	2.43	49.02	44.13	

6.4. Clinical and biochemical characteristics of study participants

Table **5** shows study participants 'quantitative clinical and biochemical variables at baseline. The mean was reported for data that were normally distributed, with the standard deviation (SD). A median was reported for data with a skewed distribution, with an interquartile range (IQR) from the 2.5^{th} and 97.5^{th} percentile.

6.4.1. Clinical data of study participants

As demonstrated in **table 5,** the maximum and minimum BMI of the study participants was 18.70 kg/m^2 and 34.4 kg/m^2, respectively with mean of 25.30 kg/m^2 and standard deviation of

3.26 kg/m^2.When we categorized the participants according to their BMI, just over half of the participants 59 (51.3%) were fallen into the category of normal BMI (18.24.9-24.9 kg/m^2), 46(40%) of them were overweight (BMI between 25-29.9 kg/m^2) and only 10(8%) were obese (BMI≥30kg/m^2). However, when they were classified according to their WC, approximately two third of them (73.9%) had visceral obesity (WC≥94 cm). Only one third of them had normal waist circumference (WC<94 cm).

Though we could not take two measurements of blood pressure to calculate the average ,a single measurements made by professional nurses and physicians during the study period for diagnosis purpose was taken properly .Based on these measurements, the median SBP was 130 mmHg with inter quartile range of the 2.5th and 97.5th percentile between 100 mmHg-180mmHg and the median DBP was 80mmHg with inter quartile range of the 2.5th and 97.5th percentile was between 60mmHg-100mmHg.Though the DBP of the majority 90 (78.3%) was in the normal range, the SBP of 68 (59.1%) of them were above the normal range. Therefore, blood pressure was not well controlled among the participants.

6.4.2. Laboratory data of study participants

Hormones

As demonstrated in (**table 5**), the mean TT was 19.15 nmol/L with SD of 6.99nmol/L. Its maximum and minimum values were 6.97 nmol/L and 37.13 nmol/L, respectively. Similarly, the mean of LH was 7.56 mIU/ml with SD of 3.30 mIU/L. The minimum and maximum values of LH were 2.71 mIU/ml and 18.7 mIU/L, respectively. The median value of LH was 7.07mIU/L with 95 % (CI 6.95-8.17). The 2.5th and 97.5th percentile distribution of LH was found to be 3.37 mIU/ml and 16.01mIU/ml, respectively before log transformation. After logarithmically normalized and the 2.5th and 97.5th percentile values were taken, and their antilog was found to be 3.39 and 15.85 mIU/ml, respectively.

The mean FSH was 7.67 mIU/ml with SD of 4.94 mIU/L. The minimum and maximum values of FSH were 1.39 mIU/ml and 26.25mIU/ml, respectively. The median was 6.31 mIU/L with (95% CI: 6.76-8.59). The 2.5th and 97.5th percentile distribution of LH was found to be 2.19 mIU/ml and 23.62 mIU/ml respectively before log transformation. After logarithmically normalized and the 2.5th and 97.5th percentile values were taken, and their antilog was found to be 2.19 and 23.44 mIU/ml .

Table 5: Baseline Clinical and biochemical characteristics of male with T2DM attending treatment at Diabetic clinic of TASTH from February to March, 2017 (n=115)

Variables	Categories	N (%)	Mean ±SD	Range	Median (IQR)
BMI (Kg/m^2)	18.5-24.9 [Normal]	59(51.3)	25.30±3.26	18.70-34.84	
	25-29.9 [Overweight]	46(40.0)			
	≥30 [Obese]	10(8.7)			
WC(cm)	<94[Normal	30(26.1)	98.56±8.70	80-118	
	≥94 [Obese]	85(73.9)			
SBP (mmHg)	<130	47(40.9)		100-190	130(100-180)
	≥130	68(59.1)			
DBP (mmHg)	<85	90(78.3)		60-110	80(60-100)
	≥85	25(21.7)			
TT (nmol/L)	≤12.1	29(25.2)	19.15±6.99	6.97-37.13	
	>12.1	86(74.8)			
LH (mIU/ml)	>7.8	43(37. 4)	7.56± 3.30	2.71 -18.7	7.07(3.37- 16.01)
	<7.8	72(62.6)			
FSH (mIU/ml)	≤14	102(88.7)	7.68±4.94	1.39-26.25	
	>14	13(11.3)			
FBS (mg/dl)				82-313	168(82.9-307.2)
TC (mg/dl)	<200	98(85.2)		72.2-253.2	148(84.1-244.8)
	≥200	17(14.8)			
TRIG (mg/dl)	<150	64(55.7)		59.4-776.4	142 (71.1-656.7)
	≥150	51(44.3)			
LDH-C (mg/dl)	<100	70(60.9)	90.53±32.46	28.5-170.6	
	≥100	45(39.1)			
HDL-C (mg/dl)	<40	83(72.2)	35.5±10.4	13.3-71	
	≥40	32(27.8)			

Serum lipids and glucose

Serum lipids and glucose of men with T2DM who participated in this study was determined to evaluate whether dyslipidemia and hyperglycemia can be risk factors for hypogonadism. The values of all these parameters are in (table 5).As indicated in the table, mean HDL-C was 35.19mg/dl with standard deviation of 10.44 mg/dl. This value was found to be below the normal range. About 72.2% of participants in this study had low HDL-C which is less than

40mg/dl. It contributed to the largest portion of the high prevalence of dyslipidemia among these patients. Its minimum and maximum value was 13.3 and 71 mg/dl.

The mean level of LDL-C among the study participant was 90.53 mg/dl with standard deviation of 32.46 mg/dl. The minimum and the maximum values among these patients were 28.50 and 170.6mg/dl, respectively .We found that about 39.1% of patients participated in this study had high LDL-C which is the major risk factor for atherosclerosis.

The minimum and maximum values of TC in this group of patients were 72.2mg/dl and 253.2 mg/dl, respectively with mean of 155.5 mg/dl and standard deviation of 39.15 mg/dl. The median was 148mg/dl with (95% CI of 148.20-162.64). About 15% of the participates had elevated level of this lipid. The 2.5^{th} and 97.5^{th} percentile distribution of this analyte among the study population was 84.10-244.81mg/dl with median of 148.1mg/dl. Then the antilog of 2.5^{th} and 97.5^{th} percentile values was computed and it was found to be 83.75 and 245.5 mg/dl respectively.

The median of value of TRIG was 141.9 mg/dl with the 2.5th and 97.5th interquartile range of 71.07- 656.65mg/dl. But its minimum and maximum values were 59.4 and 776.4 mg/dl. Similarly, the median value of FBG of the study subjects was 168 mg/dl in the interquartile range of the 2.5th and 97.5th percentile values (82.9-307.2).Its minimum value was 82mg/dl whereas its maximum value was 313mg/dl.

6.5. Prevalence of androgen deficiency symptoms, low testosterone level and secondary hypogonadism among study participants

Androgen Deficiency in Aging Male (ADAM) questioner was used to assess symptoms of hypogonadism among our study participants. As summarized in, 104(90.43%) were ADAM positive whereas the remaining 11(9.57%) were ADAM negative (**table 6**) based on the criteria of classification of responses to ADAM questioner outlined in the methodology section.

It was below our expectation to see that only 27(23.48%) of ADAM positive participants were with low TT level (TT≤12.1nmol). Among ADAM negative ones only 2(1.74%) were with low TT level. Therefore, only 23.48% of study subjects met the current definition of hypogonadism which include the presence of symptoms and low testosterone level.

Of those with low TT, 20(74.1.0%) had low or low normal LH and FSH level. Hence, they were categorized as hypogonadal patients with secondary hypogonadism (hypogonadotrophic hypogonadism).

Table 6: Frequency of low and normal total testosterone groups in ADAM positive and negative study subjects (n=115)

Response to ADAM Questioner	**Testosterone groups**		Total
	Low TT [TT≤12.1nmol/L]	Normal TT [TT>12.1nmol/L TT]	
ADAM positive	27(23.5%)	77(66.9%)	104 (90.4%)
ADAM negative	2(1.7%)	9 (7.8%)	11 (9.6%)
Total	29(25.2%)	86(74.8%)	115(100%)

6.6. Test characteristics of Androgen Deficiency in Aging Men (ADAM) questioner

Characteristics of ADAM questioner were determined based on the value in table 6. Sensitivity of ADAM questioner to identify those with low TT was very good at 93.1%. It has also good negative predictive value (81.8%). However, the specificity (10.5%) and positive predictive value (26%) was very poor as demonstrated in (**table 7**).

Table 7: The statistical measures of ADAM questioner in T2DM patients with low testosterone

Statistical measures	**Result**
Sensitivity	93.1%
Specificity	10.5%
Positive predictive value	26%
Negative predictive value	81.8%

The frequency distribution of androgen deficiency symptoms among study participants was summarized in (**table 8**). The two relatively more specific symptoms “loss of libido” which was the most common symptoms in 104 (90.4%) of the total study participants followed by

"erectile dysfunction" , the second most frequent symptoms among this population with frequency of 98(85.7%) out of 115 patients participated in this study. The frequency of the symptom "loss of height "was the least of all .

Table 8: Distributions of symptoms of hypogonadism among study participants (N=115)

No	Symptoms	Responses			
		Yes		No	
		Frequency	Percent	Frequency	Percent
1	Decreased libido	102	88.7	13	11.3
2	Lack of energy	84	73.0	31	27.0
3	Decrease in strength/endurance	86	74.8	29	25.2
4	Lost height	22	19.1	93	80.9
5	Decreased enjoyment of life	69	60.0	46	40.0
6	Often sad or grumpy	76	66.1	39	33.9
7	Erectile dysfunction	98	85.2	17	14.8
8	Recent deterioration in sporting ability	71	61.7	44	38.3
9	Falling asleep quickly after dinner	54	47.0	61	53.0
10	Deterioration in work performance	57	49.6	58	50.4

6.7. Comparison of the clinical and biochemical parameters of subjects with and without hypogonadism

Patients' history of diabetic complications (retinopathy, neuropathy, and nephropathy), history of hypertension , history of alcohol consumption, history of smoking were checked whether there were significantly difference between low TT and normal TT groups of the study participants or not by using chi-square ($\chi 2$) test. History of smoking did not satisfy chi-square assumption because two cells had expected count less than 5. All other variables were not significantly differed between the two groups ($p > 0.05$) except history of hypertension with $\chi 2\ (1) = 4.621$, P=0.032.

For parametric data presented in (**table 9**) independent sample t test was used to evaluate whether the mean differences of each indicated parameters were statistically significant (P <0.05) between hypogonadal and eugonadal groups. The mean BMI was significantly higher in hypogonadal group (26.9±3.5 kg/m^2) than in eugonadal group (24.86 ±3.02 kg/m^2), t (113) =3.035, p=0.003. Similarly, the mean WC was significantly higher in hypogonadal group (104.85±8.77 cm) than in eugonadal group (96.62 ±7.75 cm), t (113) =4.676, p<0.001. However, the mean HDL-C level was lower in hypogonadal group (31.08±8.57mg/dl) than in eugonadal group (36.83±10.63mg/dl) t(113)=-2.563,p=0.012 .Similarly the mean FSH level was significantly lower in hypogonadal group (6.28 ±2.75 mIU/ml) than in eugonadal group (8.10±5.38 mIU/ml), t (113) =-2.34, p=0.022. However, the two groups did not differ significantly in mean of LH, LDL-C and TC.

For non- parametric data demonstrated in (**table 9**), a Mann-Whitney U test was used to compare hypogonadal and eugonadal groups. A Mann-Whitney U test indicated that FBG level was significantly higher in hypogonadal group with median of 235mg/dl than in eugonadal group with median of 161 mg/dl, U=711.5, p=0.002. Similarly, TRIG was significantly elevated among hypogonadal group with median of 174mg/dl than in eugonadal ones with median of 136 mg/dl. However, there was no statistically significant difference in the age, duration of DM, SBP, and DB between the two groups.

Table 9: Comparison of clinical and biochemical characteristics of study participants between hypogonadal and eugonadal groups (N=115)

No	Parametric values	Testosterone level		P value
		Hypogonadal group (n=27)	**Eugonadal group (n=88)**	
		Mean ± SD	**Mean ± SD**	
1	BMI(kg/m^2)	26.90±3.54	24.80±3.02	0.003
2	WC(cm)	104.85 ±8.77	96.62± 7.75	<0.001
3	LH(mIU/ml)	7.06 ±3.09	7.72 ±3.36	0.365
4	FSH(mIU/ml)	6.28 ±2.75	8.10± 5.38	0.022
5	TC(mg/dl)	159.37±42.35	154.20±38.28	0.549
6	LDL(mg/dl)	86.45 ±36.09	91.78±31.38	0.458
7	HDL(mg/dl)	31.08 ±8.57	36.83±10.63	0.012
No	**Non Parametric values**	**Median**	**Median**	**P value**
8	Age(years)	60.0	60.0	0.721
9	Duration of DM (years)	6.0	7.0	0.629
10	SBP(mmHg)	130.0	130.0	0.293
11	DBP(mmHg)	80.0	80.0	0.583
12	FBS(mg/dl)	235.0	161.0	0.002
13	TRIG(mg/dl)	174.0	136.0	0.047

6.8. Comparison of variables between age and BMI categories

Kruskal-Wallis test indicated that only DM duration $\chi 2(3)=15.311$, $p=0.002$; SBP $\chi 2(3)=14.852$, $p=0.002$;LH $\chi 2(3)=9.974$, $p=0.019$ and DM duration $\chi 2(3)=15.311$, $p=0.002$ showed statistically significant difference between age categories. But the rest variables in table 7 did not show significant difference between age categories. Though the variance was not significant between age groups, mean FSH level seems increasing across age categories as shown (**figure 5 right).**

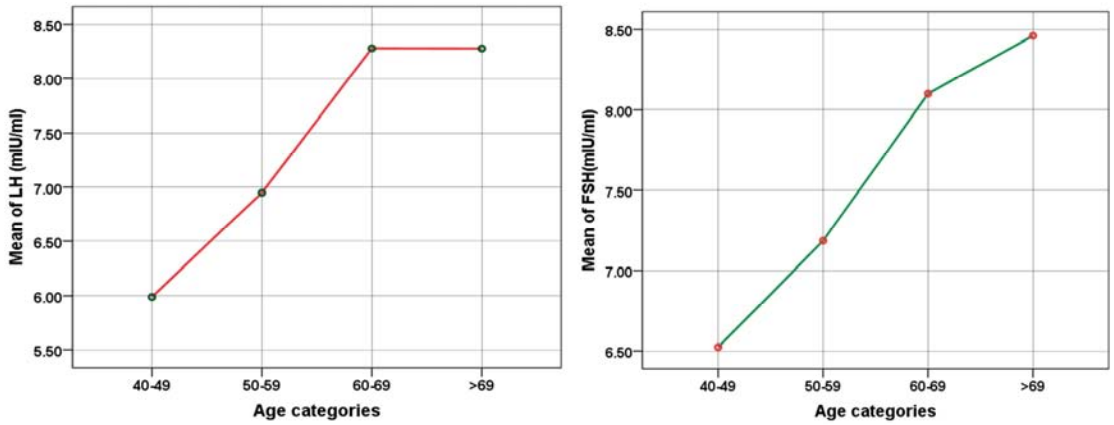

Figure 5: Mean plots of LH (left) and FSH (right) in different age categories

Analysis of variance (ANOVA) indicated that WC, SBP,DBP and TT, With F(2)= 29.5, p<0.001; F(3)=4.022 , P=0.021; F(3)=7.267, p=0.001 and F(3)=5.4740, p=0.005 differ significantly across BMI categories respectively.

To identify whether there is significant mean difference of TT between paired BMI categories, Turky's Post –Hoc analysis of honestly significant difference (HSD) was performed. The result showed that there was no statistically significant mean difference of TT between those with normal BMI and overweight ones (p=0.725) .However, significant TT mean difference was observed between those with normal BMI and obese ones (p=0.04). Similarly, significant mean difference of TT was also observed between overweight and obese ones (P=0.016) as indicated in (**figure 6**).

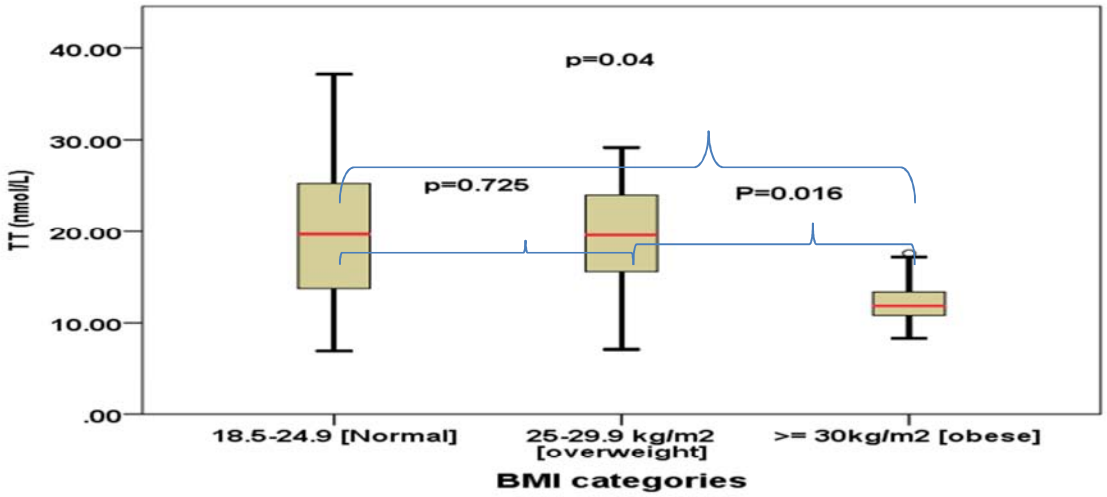

Figure 6: Box and whisker plots of total testosterone in three BMI categories

6.9. Correlation and multiple linear regression analysis result of independent variables with total testosterone

Pearson correlation analysis of TT and independent variables which include age, DM duration, BMI, WC, HDL-C, LDLC, TRIG and FBG was assessed. As depicted in **figure 7 left to right TT** is inversely correlated with WC and BMI (r=-.465, P<0.001) and (r=.363; *p*<0.001) respectively.

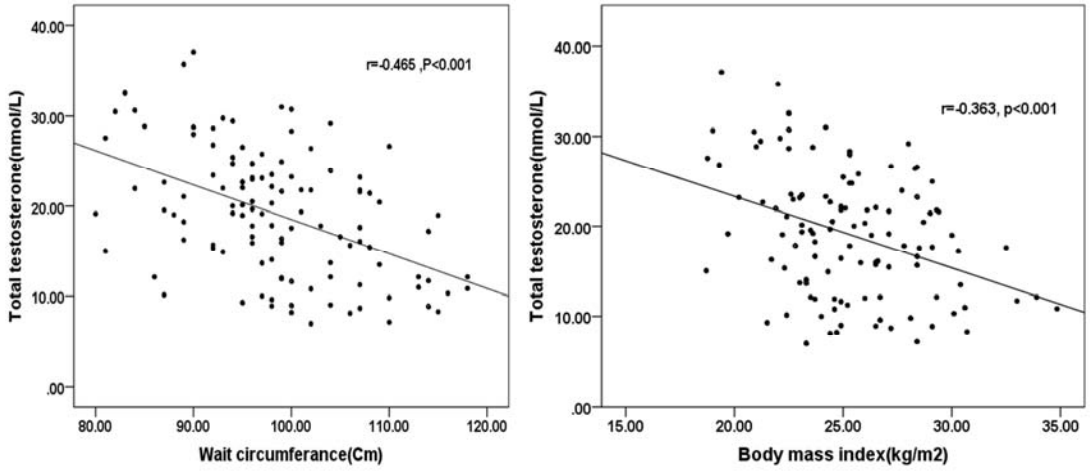

Figure 7: Scatter Plots that show relationship between TT and WC (left), TT and BMI (right)

Similarly, as shown in **figure 8** from left to right TT was significantly inversely correlated with FBS with (*rho*=-.328, p<0.001) and TRIG (*rho*=-.357,p<0.001) respectively. But it was significantly positively correlated with HDL-C (*r*=.339, *p* <0.001) as indicated in (**figure 9).** However**,** the level of this hormone was not correlated with age, DM duration, SBP, DBP, TC, and LDL-C with p values of 0.874, 0.616, 0.100, 0.185, and 0.364 respectively. It was unexpected to see that TT was also not significantly correlated with LH and FSH with *P*=0.164 and 0.488 respectively.

However, by stepwise multiple linear regression analysis only WC and FBG remained statistically significant independent predictors of hypogonadism in men with T2DM participated in our study.

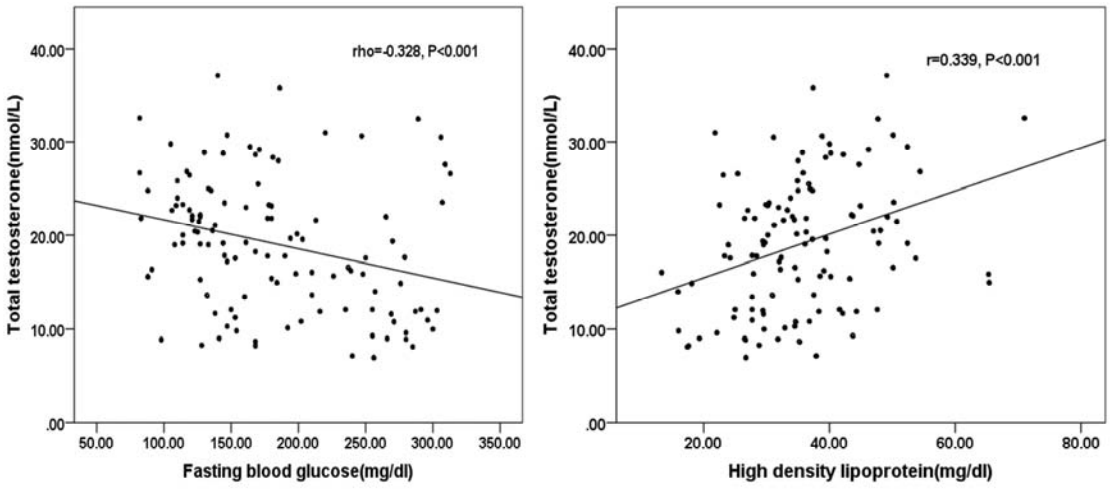

Figure 8 : Scatter Plots that show relationship between TT and HDL-C (left) and TT and FBG (right)

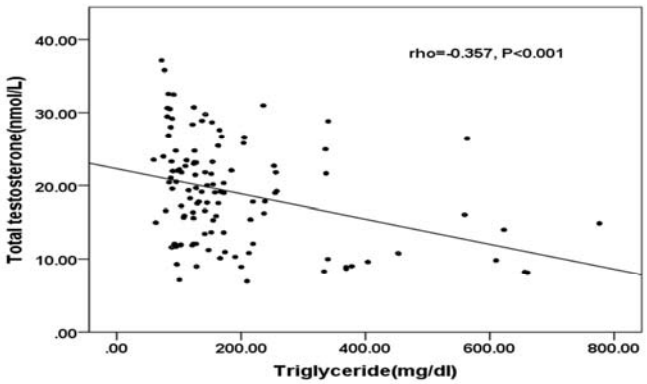

Figure 9 : A Scatter Plot that show relationship between TT and TRIG

6.10. Multiple logistic regression analysis result of factors associated to hypogonadism

Simple logistic regression was performed for TT and independent variable and summarized in (**table 10**).Then; those with a p-value < 0.25 were entered into a multivariate model with automatic backward and forward likelihood ratio method of elimination, based on the p-values in the model. Variables were dropped if the p-value was non-significant (< 0.05). A multiple logistic regression was then run treating hypogonadism as the dependent variable and WC, BMI, FSH, FBG, HDL-C and TRIG as independent continuous variables. The rest variables were dropped because their p value was greater than 0.25.

Table 10: Binary Logistic regression analysis result of hypogonadism and independent variables

Variables	β –value	SE	Wald	P-value	Exp (β)	95% CI for Exp (β)
Age	0.002	0.022	0.009	0.926	1.002	0.961-1.045
BMI*	0.166	0.069	5.80	0.016	1.181	1.031-1.351
WC*	0.096	0.029	11.10	0.001	1.100	1.04-1.164
SBP	0.008	0.011	0.62	0.432	1.009	0.987- 1.030
DBP	0.009	0.022	0.15	0.694	1.009	0.966 - 1.053
DM duration	-0.005	0.032	0.02	0.887	0.995	0.935- 1.059
LH	-0.081	0.072	1.25	0.267	0.922	0.8-1.063
FSH*	-0.112	0.060	3.49	0.062	0.0894	0.795-1.006
FBS*	0.015	0.004	16.50	<0.001	1.015	1.008-1.023
TRIG*	0.003	0.001	4.99	0.025	1.003	1.000-1.006
HDL-C*	-.055	0.024	5.11	0.024	0.947	0.903-0.993
LDL-C*	-0.007	0.007	1.20	0.274	0.993	0.979-1.006
TC	0.002	.005	0.12	0.731	1.002	0.991- 1.013

*Indicate variables chosen to run multiple logistic regressions

The model was then checked using Receiver Operating Characteristics (ROC) whether it can correctly discriminate low TT group from normal TT group. The area under the curve was 0.841 (CI: 0.753-0.929) . This area is greater than 0.7. So it was taken as good model fit to the data. The model can correctly classify 82.6% of the data.

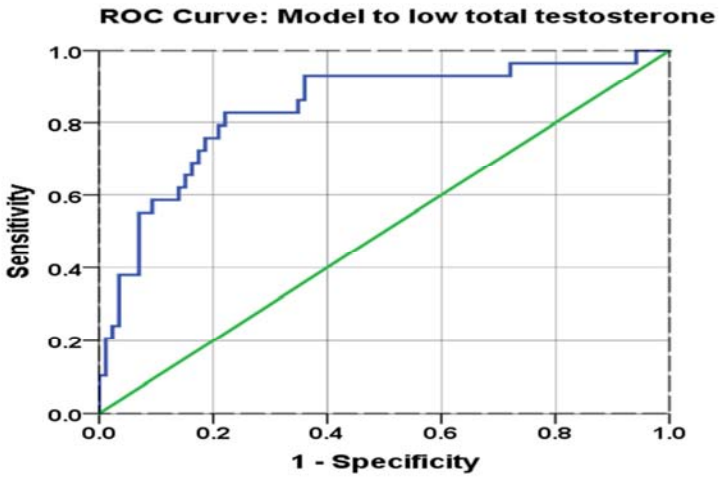

Figure 10 : Multiple logistic regression ROC curve model for low total testosterone

Finally, only WC and FBG remained independent risk factors of hypogonadism. Table 11 indicated that 1 cm increase in WC increase odds of developing hypogonadism by 1.2 times, while controlling for FBG. Similarly, an increase in FBG by 1mg/dl increases odds of developing hypogonadism by 1.02 times while controlling for WC.

Table 11: Multiple logistic regression analyses result of predictors of hypogonadism in T2DM subjects (N=115)

Variables	B	S.E.	Wald	P value	Odd ratio	95% C.I.for EXP(B)	
						Lower	Upper
WC	0.154	0.038	16.484	0.000	1.20	1.08	1.256
FBG	0.015	0.004	11.774	0.001	1.02	1.01	1.024
Constant	-19.614	4.294	20.863	0.000	0.000		

7. Discussion

We assessed hypogonadism and its associated risk factors among 115 Ethiopian men with T2DM attending treatment follow up at TASTH. It was the first study performed in this population of men at this tertiary setting aiming to determine prevalence of symptomatic hypogonadism, hypogonadotrophic hypogonadism, and its associated risk factors and compared differences in independent variables in low TT and normal TT groups of this population.

Prevalence of hypogonadism: Prevalence of low serum testosterone in men with T2DM varies from 20 to 64% depending on the population and whether total or free testosterone is used to make the diagnosis [18]. In this study about ninety percent of T2DM male patients were with androgen deficiency symptoms or ADAM positive when assessed with ADAM questioner. The most common symptoms were loss of libido (90.4%) followed by erectile dysfunction (84.7%). A Cross-sectional study has found a high prevalence of low libido (64%), erectile dysfunction (74%), and fatigue (63%) in hypogonadal men with T2DM. However, the presence of these symptoms was similarly high in eugonadal men with T2DM as well (48, 65, and 57%) respectively [25].

Using recent definition of hypogonadism and testosterone cut off value of International Society of the Aging men, only 27(23.5%) with hypogonadism. This prevalence hypogonadism was lower than those reported in the literature even though there are differences in cut off value of TT, age categories, study design, and laboratory methods among them. A similar cross sectional study conducted in South Africa on 150 male T2DM patients with age greater than 50 years, reported very close prevalence of androgen deficiency symptoms (94.7%) as in our study. Unlike our study, it identified higher (50%) prevalence of low TT among these men with a lower cut off value (TT<9.9 nmol/L) [43]**.** This might be due to the non-specific nature of the components of the ADAM questionnaire which may be responsible for its poor overall efficiency in identifying male androgen deficiency **[47] .**A similar study in Egypt conducted on smaller sample size of 70 T2DM male patients with age ranged from 30-50 years reported a lower prevalence (58.6%) of androgen deficiency symptoms but higher (40%) prevalence of low TT (TT< 8 nmol/L) [48] when compared to our study. In another similar study conducted in Egypt on 140 samples of men with T2DM,

34.2% of them were hyopogonadal [49] .This was also higher than that of ours. This might be due to the higher mean BMI of study participants in these studies as compared to the mean BMI in our study. Because, obesity is confirmed to be important contributor to the higher prevalence of hypogonadism among men with T2DM [18] . However, a study conducted in Iran on smaller sample size of 85 T2DM patients between age of 40-60 years reported lower prevalence of hypogonadism (11.8%) based on TT level (Cut off not mentioned) compared to our study but higher prevalence based on calculated free testosterone (36.6%),and bioavailable testosterone (35.3%) [50]. In contrast to Iranian study, a study conducted in New york on 103 T2DM patients reported lower prevalence of hypogonadism (33%) measuring free testosterone or calculated free testosterone, but higher prevalence (43.7%) using TT levels less than 10.4 nmol/L [21].This shows that there are inconsistencies among research findings with regards to prevalence of hypogonadism in this group of population.

Other cross sectional studies conducted on larger sample sizes as in a study in China [15] and Korea [51] with the same cut off value of TT with that of our study (TT≤12.1 nmol/L or 350ng/dl) also reported higher prevalence of hypogonadism 35.2% in samples of 213 and 34.9% in samples of 464 T2DM respectively. But, a study conducted in England reported a lower prevalence of 17% with TT<8nmol/L and 31% with TT between 8-12 nmol/L in samples of 359 study subjects with age ranged 32-83 years. But this is entirely due to variation in TT cut off values. If those with TT between 8-12nmol/L were also considered hypogonadal as we did, the prevalence of hypogonadism would rise to 51% .This study also reported 50% prevalence of hypogonadism among the subjects with free testosterone [25]. Unexpectedly higher prevalence (78%) among Sudanese men with T2DM was reported in a recently published comparative cross sectional study in Sudan on 300 men with T2DM and 100 controls .Hypogonadism was solely defined as low TT below 10.41nmol (300ng/dl)[44].

Therefore, the possible explanation why hypogonadism was less prevalent among T2DM patients attending follow up treatment at TASTH might be due to infrequency of obesity as mentioned earlier among these men. As compared to participants in aforementioned studies, men with T2DM participated in our study had relatively lower mean ±SD of BMI and WC of 25±3.25 and 98.56±8.70 respectively. For instance, the mean ± SD of BMI and WC of that of South Africans was 30.7±5.37 and 112±16.2, respectively [48]. Additionally, the mean ±SD of BMI and WC of participants of the study in Egypt were 32.87 ±5.648 and 113.96 and 113.96 ± 18.25) respectively [49]. These differences in BMI which is an indicator of general

obesity and WC which is indicator of central or visceral obesity might contribute to the big differences in prevalence of hypogonadism among our study participants and those in specified studies. Because, it has been suggested that an excessive increase in fat mass may result in an increase in the activity of aromatase enzyme which causes greater conversion of testosterone into estradiol . An increase in estradiol levels would lead to the suppression of gonadotrophin releasing hormone and impaired secretion of gonadotropin by the pituitary gland. This results in the reduction of both testosterone secretion and mature sperm production [18,19]. This confirms that obesity represents an important confounding factor in the relationship between testosterone and T2DM. Obese men and men with T2DM can have secondary hypogonadism because of the peripheral and central insulin resistance and the effect of pro-inflammatory cytokines (TNFα and IL-6) on the hypothalamic pituitary-gonadal axis [2]. Increase in SHBG with age may also contribute to elevated TT in our study population as majority of them were above the age of 60 years [35]. But this finding was challenged by a study conducted in England [25].

As summarized in the recent publication of international society of aging male guideline, testosterone deficiency symptoms particularly loss of libido or vigor may also be seen with TT levels as high as 15 nmol/L .It also forwarded that a state of elevated LH in the presence of normal testosterone but with hypogonadal symptoms should be considered as hypogonadism [4].Most studies including ours did not consider this evidence in defining hypogonadism .We thought this might be another reason why symptoms of hypogonadism and testosterone deficiency did no correspond to each other as expected.

Test characteristics of Androgen Deficiency in aging men questioner: Our study determined test Characteristics of ADAM questioner. It had very good sensitivity (93%) and negative predictive value (81.8%) of ADAM questioner to identify those with low TT. However, it had very poor specificity and positive predictive value. In a study conducted in Nigeria, performances of the ADAM questionnaire for the clinical detection of androgen deficiency in black Sub-Saharan African men with T2DM were evaluated. The study determined 88.1%, specificity of 44.7%, PPV of 50.0%, NPV of 85.7% [47]. A study in South Africa reported very high sensitivity (95%) and very low specify (5%) as in our study to identify those with low total testosterone. This study also reported very good sensitivity (100%) and negative predictive value (100%) in those with low calculated free testosterone [43]. Both aforementioned studies concluded that ADAM questioner cannot be used as a

surrogate for biochemical determination of serum testosterone in evaluation of hypogonadism in T2DM.

Prevalence of secondary hypogonadism: According to the recommendation of International society of Aging Male [2] and International Endocrine Society [4], measurements of serum LH and FSH assists in differentiating between primary and secondary hypogonadism. Based on these recommendations we assessed prevalence of secondary (hypogonadotrophic hypogonadism). It was 74.10% taking TT≤12.1nmol, FSH<14 mIU/ml and LH<7.8mIU/ml level. The rest 25.9% had primary hypogonadism with elevated FSH or LH. A similar study in in Jordan also reported a higher prevalence (83.1%) of secondary hypogonadism and 16.9% primary hypogonadism [39]. Another study in Nigeria observed that 76.3% of subjects with overt hypogonadism (TT<8nmol/L) and 89.1% of patients with possible hypogonadism TT between (8-12 nmol/L) were hypogonadotrophic hypogonadal. Elevated gonadotrophins were noted in 23.7% and 10.9% of subjects with overt and possible hypogonadism, respectively [40].

The association between serum TT and gonadotropin was not clear in males with T2DM. However, different studies forwarded that insensitivity to insulin at the hypothalamic level may contribute to the decrease in GnRH which in turn decreases the level of gonadotrophins (LH and FSH) [10]. On the other hand, obesity can promote estradiol secretion and suppress hypothalamic GnRH production with negative feedback mechanism [18].

Association of TT level with independent variables: We observed that age, duration of diabetes, none of diabetic complications, monthly income, and history of alcohol consumption were not significantly associated with TT. In contrast to our study, a study in Jordan showed a significant relationship between age, income and neuropathy with TT [39]. A possible explanation given for the association of income and testosterone level was that a lower monthly income may function as a marker for poorer access to health, increased stress, and adverse health behaviors. However, cost free access to health facilities for those who cannot afford might be the reason for the lack of association between income and testosterone level in our study. Unlike our study, a study in Nigeria reported significant associations between alcohol histories and low TT levels [29].This might be due to the difference in testosterone cut off values between the two studies. However, an Indian study reported similar result with ours [41].The correlation of age with TT level was inconsistent. Some

studies showed negative correlation between age and TT level [25, 40]. The most possible explanation is that SHBG which accounts for 60-80% of testosterone binding rises with age and may serve as a confounding factor when TT is used solely in the evaluation of testosterone levels. Low levels of SHBG on the other hand may occur in the presence of insulin resistance; thus, resulting in TT levels. In the absence of the assessment of bioavailable testosterone levels, the degree to which this confounder- SHBG- affected our results if at all is difficult to speculate on [29] .However, study in New York and south Africa did not observe significant correlation between TT and age as in our study [21,43].

Our study identified BMI, WC, FBG and TRIG were negatively and significantly correlated with testosterone level where as HDL-C was positively correlated with it. In line with our study, a case control study in Ghana revealed that TT level was inversely related to BMI, FBG and TRIG among T2DM groups [42]. However, the level of this hormone was not correlated with DM duration, SBP, DBP, TC, LDL-C, LH, and FSH in our study participants. Lack of association between LH/FSH and TT might be due to the rise of these hormones slightly with age, as observed in our study and others suggesting an age-related alteration in this feedback mechanism [21]. Additionally significant number of the study participants had primary hypogonadism (25.9%) which is related to testicular problem rather than to gonadotropins. Though, FBG was related to the level of TT in a Korean study, BMI was not significantly related with it [50].

These associations can be indirectly explained as testosterone replacement therapy was found to alter some of the variables. As summarized in Endocrine society update of hypogonadotropic hypogonadism in T2DM and obesity, a decrease in WC of 14 cm in men with new onset T2DM treated for 1 year with transdermal testosterone, diet, and exercise. The control group that was prescribed only diet and exercise lost 5 cm. A decrease in 1.63 cm in WC after one month testosterone treatment was also reported as this update noted .The update also outlined that, BMI did not change in any of the studies despite the decrease in abdominal girth [5].Nature review of Endocrinology forwarded that TRT were found to decrease TRIG, levels TC and LDL-C. The review also noted that TRT improves glycemic controls in interventional studies but its action on HDL-C was inconsistent [5].

Comparison of independent variables in low TT and normal TT groups of T2DM patients: In our study age, duration of diabetes, SBP and DBP, LH, FSH, LDL-C and TC did not differ significantly between hypogonadal and eugonadal groups. But, WC, BMI, FBG and TRIG were significantly higher inhypogonadal group as compared to eugonadal group whereas HDL-C was significantly lower in the former one. A similar study in Italy partly reported the same result with regards to HDL-C, TRIG, age, HDL-C, TRIG, and BMI but not with duration of diabetes, LH, and FSH [31]. However, a cross sectional study in china did not report significant difference in the level of FBG, TG and HDL-C between these groups. Age, diabetic duration, FSH, LH, was not significantly differ between hypogonadal and eugonadal groups of T2DM patients in a study conducted in Egypt [49] .This result was in line with ours. Unlike our study, all lipid profile was not significantly differed between the two groups. On the contrary to our study FBG, TRIG, and HDL-C levels were not significantly different in hypogonadal and eugonadal groups as a study in china revealed. Though this study reported similar finding with regards to age, diabetes, duration, diabetic complications, DBP, SBP, TC and LDL-C, it reported that the two groups significantly differ in gonadotrophin levels which was actually inconsistent with our findings [15].This lack of association might be partly due to the existence of significant percentage (25.9%) of hypogonadal men with primary hypogonadism in participated in our study which is actually related to gonadal problem but not with deficiency of gonadotrophins.

Risk factors of hypogonadism: According to result of multiple logistic regression analysis of the association between independent variables and TT levels, only WC and FBG remained associated risk factors of hypogonadism. From this analysis we confirmed that WC was a better risk factor for hypogonadism than BMI in our study population. We thought HDL-C which was less than 40mg/dl in majority of our participants could be other risk factors for hypogonadism. But the relationship between testosterone and HDL-C might be confounded by the fact that both HDL-C and testosterone inversely related to BMI. BMI was also positively associated with WC. Due to multi collinear nature of these variables they failed to be strong predictors of hypogonadism in our study population. In fact, a practical guide to male hypogonadism in the primary care setting summarized that TRIG levels are negatively linked with TT but HDL-C levels are positively linked to testosterone levels in middle-aged men [2].

Our finding regarding FBG as a risk factor was in line with a research conducted in Ghana and korea .This can be explained by the fact that the association between glycaemia and reduced TT concentrations may be an effect of glycaemia on the testicular microvasculature. Thus, glycemia alters Ledig cell function, directly causing primary hypogonadism. In addition, if glucose is not reaching the cells because of insulin insensitivity, there will not be enough energy generated for the various metabolic processes involved in maintaining testosterone levels [42, 50].

Additionally, chronic hyperglycemia and hyperinsulinemia are associated with hypogonadism, and low testosterone levels may play a role in the development of T2DM,but the impact of more acute changes in glucose and/or insulin on the hypothalamic pituitary testicular axis is less well understood. Manipulation of the glucose milieu in clinical scenarios may also influence testosterone levels. It has been demonstrated that glucose administration impacts TT levels in men without preexisting androgen deficiency with implications for the biochemical categorization of androgen status [52].

Clinically, visceral adiposity is measured by WC or waist-to-hip ratio [46]. Testosterone is metabolized to estradiol, primarily in adipose tissue by aromatase, which has increased activity as visceral adiposity increases. Estradiol directly feeds back and inhibits the hypothalamic–pituitary–testicular axis and this result in decreased testosterone [10].This can be clear evidence why increase in WC identified as a risk factors of hypogonadism in our study and others.

Visceral adiposity also enhances delivery of free fatty acids to the liver leading to a reduced hepatic insulin clearance and further increase circulating insulin levels leading to hyperinsulinemia. Free fatty acids then accelerate gluconeogenesis and triglyceride synthesis by the liver, increasing esterification of free fatty acids and reduced hepatic degradation of apolipoprotein B, resulting in increased synthesis and secretion of small very low density lipoprotein particles. The increase in very low density lipoprotein leads to clinical hypertriglyceridemia, another component of the metabolic syndrome. In addition to the effects on the liver, the increase in free fatty acids decreases peripheral glucose disposal primarily in skeletal muscle and this result in hyperglycemia [18].This hyperglycemia in turn has an effect on testicular microvasculature and resulted in decrease of testosterone as mentioned before.

8. Strength and limitations of the study

8.1. Strength of the study

This study did not solely relied on testosterone level to define hypogonadism as some studies in Africa and other parts of the world did. It thoroughly assessed symptoms of hypogonadism using well known ADAM screening questioner and determined the prevalence of androgen deficiency symptoms.

The study also incorporated major clinical data from measurements, medical records, using questioners and extensive laboratory measurements to evaluate risk factors associated with low testosterone. Measurements of height, and weight to calculate BMI, WC were taken by professional nurses.

Selection bias was minimized because consecutive patients who met the criteria were selected. This bias might have occurred if large percentage of patients refused to participate. But none of refused the consent to take part in the study.

Moreover, hormones and lipid profiles were determined in accredited referral clinical chemistry of Ethiopian Public Health Institute.

8.2. Limitations of the study

This study was conducted on small sample size in a tertiary referral outpatient diabetic clinic where most of the patients had many complications and numerous comorbidities. Because of this fact, results of this study cannot therefore be generalized to the majority of men with T2DM who follow up at primary health care.

Some of the information like the use of glucocorticoids, liver disease, and cancer were subjectively obtained from medical records and patients. Diabetic complications may be underestimated because the data were obtained from medical records.

Moreover, our inability to measure FT which is the gold standard and SHBG to calculated FT or bioavailable testosterone due to unavailability of these assays in our practice and cost respectively .We could not measure Hemoglobin A1C which is a better glycemic control indicator due to financial problem.

Conclusions

In conclusion, this cross sectional study indicated that symptoms of hypogonadism though nonspecific are prevalent in the majority of men with T2DM attending treatment follow up at diabetic clinic of TASTH. Loss of libido and erectile dysfunction are the most common symptoms in this group of population.

Despite an impressive sensitivity, the low specificity and positive predictive value of the ADAM questionnaire makes it unreliable for the detection of hypogonadism among this group of men.

Prevalence of hypogonadism was 23.5% and it is lower than those reported in the literature.

Hypogonadotrophic hypogonadism occurred in approximately three fourth of the study participants but primary hypogonadism occurred in one fourth of them.

BMI, WC, FBG and TRIG are inversely associated with TT level where as HDL-C is positively associated with it.

Mean BMI, WC, FBG, TRIG are significantly higher in hypogonadal group than in eugonadal groups of Ethiopian men with T2DM attending treatment follows up at TASTH. However, mean HDL-C is significantly lower in the hypogoadal group than in eugonadal group.

Though not noble, this study demonstrated that visceral obesity and poor glycemic control are independent risk factors for hypogonadism.

Recommendations

Based on our findings, we would like to put the following recommendation:

1. ADAM questioner should be complimented with biochemical assessment of testosterone in the diagnosis of hypogonadism because of low specificity of the questioner.
2. Even though the reported prevalence of hypogonadism was lower than those reported in the literature, it is still significant and therefore, needs special attention of endocrinologists and health care policy makers.
3. Assessment of testosterone levels in men with T2DM who had visceral obesity and poor glycemic control may help in early detection of hypogonadism.
4. The presence of high prevalence of symptoms of hypogonadism but low prevalence of testosterone deficiency in this study necessitates repeating this research with more specific tests which include measurement of free testosterone or calculated free and bioavailable testosterone by measuring SHBG. Further interventional and longitudinal study should also be conducted to evaluate the benefit of testosterone replacement therapy among hypogonadal men with T2DM.

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Annexes

Annex I: English version of participant information sheet and informed consent.

Addis Ababa University College of Health Sciences Department of Medical Laboratory Sciences

Participant information sheet

You are invited to participate in a study to be conducted by MSc student Sisay Teka at Addis Ababa University, College of Health Sciences and Department of Medical Laboratory Science. Please read the following statements and ask any unclear points before you agree to participate.

Introduction: The topic of this study is "Assessment of hypogonadism and its associated risk factors among men with T2DM attending diabetic clinic of TASTH". Participation in this study is exclusively voluntarily. If you are not interested to participate, there will be no consequences.

What is expected from me as participant of the study?

As a participant of this study, you will be asked to give 5ml of blood sample, which will be used to determine serum TT, LH, FSH, FBS, HDL-C, TG, total cholesterol and LDL-C. Your will also let us measure your waist circumference, weight and height. You are also expected to respond to questions included in the questioner and interviews which will be used as relevant data for this particular research.

Potential benefits to participant and/ or to the society

You will get laboratory test result of total testosterone, luteinizing hormone and follicle stimulating hormone which are costly for free. The study will determine prevalence of symptomatic hypogonadism and knowing prevalence is important to the clinician to look for symptomatic hypogonadism in men with T2DM and carryout appropriate management. It will also identify major risk factors for hypogonadism which correlate to T2DM to facilitate prevention, early diagnosis, and early treatment as some of them are modifiable. Additionally the study will recommend inclusion of these hormones in a routine test for men with T2DM presenting symptoms of hypogonadism. Hence, you are directly or indirectly benefiting yourself, other patients and the society at large.

Compensation for participation: You will not receive any payment for your participation in this research study.

Confidentiality: On the request paper your name or your identities will not be mentioned. Samples and information given by the participants will serve only for this research not for any other purpose.

Person to contact: Please direct any questions you may encounter during this study to the principal investigator.

Sisay Teka : **Email: sisayteka@gmail.com or mobile +25132144209**

Department of Medical Laboratory Sciences, College of Health Sciences

Addis Ababa University: Cell phone: +251- 09 32 14 42 09

Department of Medical Laboratory Science research ethics office +251 11 275 5170

English version of Informed Consent form

This page contains an agreement signature to participate in the study entitled "Assessment of hypogonadism and its associated risk factors among diabetic type 2 patients attending diabetic clinic of Tikur Anbesa Specialized Teaching hospital, Addis Ababa Ethiopia." So please read the following points and sign your signature at the end in the space provided.

1. I understood the objective of the study in "Assessment of hypogonadism and its associated risk factors among diabetic type 2 patients attending diabetic clinic of TASTH"
2. I know that the left over sample (blood) that I gave is going to be used for this study only.
3. I understand that, all the information and the results are confidential.
4. I understand that I will not get any money for my participation.
5. All the information is explained by Principal investigator.

Therefore, with full understanding of the situations I agree to give blood for laboratory analysis and fill questioners completely.

Signature of the participant: _____

Address of the participant: _____

Date: _____

Annex II: Amharic version of Participant Information sheet and consent form

በአዲስ አበባ ዩኒቨርሲቲ፤ የጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ት/ክፍል

በአዲስ አበባ ዩኒቨርሲቲ፤ የጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ትምህርት ክፍል በሁለተኛ ዲግሪ ተማሪ የመመረቂያ ጥናት ላይ እንዲሳተፉ ተጋብዘዋል፡፡ እባክዎ በዚህ ጥናት ላይ ከመሳተፍዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ጽሁፍ በጥሞና ያንብቡና / ይመልሱ ፤ ግልፅ ያልሆነ ነገር ካጋጠመዎት ይጠይቁ፡፡

መግቢያ

የጥናቱ ርዕስ “ሁለተኛው ዓይነት የስኳር ሕመም ካለባቸዉ ወንዶች ዉስጥ የቴስቶስቴሮን ሆርሞን ማነስ ጋር የሚያያዙ የወሲብ ጤና ችግሮች፤ ምልክቶች እና ተያያዥ አስጊ ሁኔታዎችን ይገመግማል”:: እርስዎ በዚህ ጥናት ላይ የሚኖሮት ተሳትፎ ሙሉ ለሙሉ በበጎ ፈቃደኝነት ላይ የተመሠረተ ነዉ፡፡ በዚህ ጥናት ዉስጥ ላለመሳተፍ ከወሰኑ በዚህ የህክምና ቦታ ዉስጥ የሚሰጥዎት አገልግሎት አይቋረጥም፡፡ በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጽሑፍ ወይም በጣት ፊርማዎትን ማስቀመጥ ይጠበቅቦታል፡፡

የጥናቱ ተሳታፊ በመሆኔ የሚጠበቅብኝ ምንድን ነዉ?

በዚህ የጥናት ውስጥ ለመሳተፍ ፈቃደኛ ከሆኑ 5 ሚሊ (አንድ የሻይ ማንኪያ የሚሆን) የደም ናሙና ይሰጣሉ፡፡ ይህም የደም ናሙና በደምዎ ውስጥ የሚገኘውን ቴስቶስትሮን ፤ፎሊክል ኢስቲሙሌቲግ ሆርሞን ፤ ሉተናይዚንግ ሆርሞን ፤ የስኳር ፤የስብ እና የኮለስቴሮል ዓይነቶችን መጠን በላቦራቶሪ ምርመራ ለማወቅ ይረዳል፡፡የወገብዎት ስፋት፤ቁመትዎ እና ክብደትዎ እንዲለካ ይጠየቃሉ፡፡ ለጥናቱ መረጃ የሚሰጡ በመጠይቁ ውስጥ የተካተቱትን ጥያቄዎች ይመልሳሉ፤ የወሲብ ጤናዎ ሁኔታ በሃኪም ለሚቀርብሎት ቃለ መጠይቅ ትክክለኛውን መረጃ ይሰጣሉ፡፡

በዚህ ጥናት ውስጥ መሳተፍ የሚያስገኛቸው ጥቅሞች

1.የላቦራቶሪ ምርምራ ዋጋቸው ውድ የሆኑትን የወሲብ ጤና ሁኔታ የሚያሳዩ ሆርሞኖችን ጥናቱ ሲያልቅ በነፃ ያገኛሉ፡፡

2.የቴስቶስቴሮን ሆርሞን ማነስ እና የወሲብ ጤና ችግሮች ምልክቶችን ስርጭት ጥናቱ ስለሚጠቁም ሃኪሞች ችግሩን ትኩረት ሰጥተው እንድያዩና የተከታታይ ህክምና አካል አድርገው መፍትሄ እንዲፈልጉለት መረጃ ይሰጣል፡፡

3. ከዚህ የወሲብ ጤና ችግሮች ጋር ተያያዥነት ያላቸው አስጊ ሁኔታዎችን ለይተው ሳይባባሱ ለመከላከል እና ለማከም ይረዳል፡፡

4. ጥናቱ የወሲብ ጤና ችግሮች ምልክቶችን ለሚያሳዩ የሁለተኛ ዓይነት የስኳር ሕመም ያለባቸዉ ወንዶች ዉስጥ በህክምና ክትትላቸው ወቅት የቴስቶስቴሮን ሆርሞን መጠን እነዲለካላቸው ለሆስፒታሉ ይጠቁማል፡፡

ስለዚህ በጥናቱ በመሳተፍዎ በቀጥታም ሆነ በተዘዋዋሪም መንገድ ለራሶ፤ ለሌሎች ህመማን ብሎም ለህብረተሰቡ ይጠቅማሉ ማለት ነው።

በዚህ ጥናት ውስጥ በመሳተፍዎ የሚከፍሉት ወይም የሚከፈሎት ክፍያ የለም፡፡

የተሳታፊዎችን ምስጢር ስለመጠበቅ: በመጠየቂያው ወረቀት ላይ የተሳታፊዎች ስም ወይም ማንነት ኣይገለጽም። በተሳታፊዎች የሚሰጥ ናሙና ለዚህ ጥናት ጥቅም ብቻ የሚያገለግል ይሆናል።

ጥያቄ ካለዎት: ይህን ጥናት በተመለከተ ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ ችግሮች ወይም ጥያቄ በሚከተለዉ አድራሻ ይጠቀሙ፡፡

ስም፡ ሲሳይ ተካ

የሕክምና ላብራቶሪ ሳይንስ ት/ክፍል፤ የጤና ሳይንስ ኮሌጅ ፤ አዲስ አበባ ዩኒቨርሲቲ

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የስምምነት መጠየቂያ ቅጽ

የጥናቱ ተሳታፊ ካርድ ቁጥር፡_____

የዚህ ጥናት ርእስ “ሁለተኛው ዓይነት የስኳር ሕመም ካለባቸዉ ወንዶች ዉስጥ የቴስቶስቴሮን ሆርሞን ማነስ ጋር የሚያያዙ የወሲብ ጤና ችግሮች ምልክቶች እና ተያያዥ አስጊ ሁኔታዎችን መገምገም ’’ ነው፡፡ ጥናቱ የሚካሄደው በጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል ይሆናል። እባክዎትን ከዚህ በታች የተዘረዘሩትን ነጥቦች በጥሞና ያንብቡና በመጨረሻ በተሰጠው ክፍት ቦታ ይፈርሙ::

1. በጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል የሚካየሄደውን የዚህን ጥናት ዓላማ ተረድቻለሁ፡፡
2. የምሰጠውም ናሙና ለዚህ ጥናት ብቻ እንደሚዉል አውቂያለሁ።
3. ለጥናቱ የምሰጠው ናሙና እንዲሁም ውጤቱ በምስጥር እንደሚያዝ ተረድቻለሁ።
4. በጥናቱ በመሳተፌ የሚከፈለኝ ክፍያ እንደሌለ ኣውቂያለሁ።
5. ሁሉም የሚያስፈልገው ነገር በተመራማሪው ይብራራልኛል።

ስለዚህ ከላይ የተጠቀሱትን ነጥቦች በመረዳት የደም ናሙና እና ለጥናቱ አስፈላጊዉን መረጃ ለመስጠት ተስማምቻለሁ።

የተሳታፊ ፊርማ፡_____

ቀን፡_____

Annex III: Questioner

ADDIS ABABAUNIVERSITY COLLEGE OF HEALTH SCIENCE SCHOOL OF ALLIED HEALTH SCIENCE DEPARTEMENT OF MEDICAL LABORATORY SCIENCE

Instruction

I. Please write your hospital Card number here _____

II. Please try to answer all

Part Ia: Socio-demographic characteristics

Instruction: Please write your choice in the space provided in response column and write shortly for items that requires you to give short answer

1. How old are you? Age (years) _____

2. How is your marital status? A. Married B. Single C. Divorced D. Widowed

3. Highest level of education? A. Informal/No B. Primary C. Secondary

D. College/university

4. Current employment? A. Farmer/peasant B. Government employee C. Private Sector employee D. Self-employment F. Unemployed

Part IIa: Questions for patients to assess risk factors for hypogonadism

5. Is there family history of T2DM? A. Yes B. No

6. Is there family history of hypertension? A. Yes B. No

7. Are you known hypertensive? A. Yes B. No

8. Have you ever smoked cigarettes? A. Yes B. No C. Stopped

9. If yes, for how long? _____years

10. If yes, how much _____/day (quantify)

11. Do you take alcohol? A. Yes B. No C. Stopped

Part Ib: Amharic version of socio-demographic characteristics questioner

እባክዎ አጭር መልስ ለሚፈልጉ ጥያቄዎች መልስዎትን ይጻፉ ። ምርጫ ላላቸው ደግሞ የመረጡትን መልስ ያክብቡ።

1. ዕድሜዎት ስንት ነው?_____

2. የትዳርዎ ሁኔታ? ሀ. ያገባ ለ. ያላገባ ሐ. የፈታ መ. በሞት የተለየ

3. የትምህርት ደረጃዎ?

ሀ. ኢ-መደበኛ/የልተማረ ለ. አንደኛ ደረጃ ሐ. ሁለተኛ ደረጃ መ. ኮሌጅ/ዩኒቨርሲቲ

4. የስራዎ ሁኔታ?

ሀ. ገበሬ ለ. የመንግስት ሰራተኛ ሐ. የግል ድርጅት ሰራተኛ መ. ነጋዴ ሠ. የግል ስራ ረ. ስራ አጥ

Part IIb: ለቴስቶስቴሮን ሆርሞን ማነስ እና ለወሲብ ጤና ችግሮች ምልክቶች መከሰት አስጊ ሁኔታዎችን ለመለየት የሚረዱ ጥያቄዎች

5. በቤተሰብዎ ሁለተኛ ዓይነት የስኳር ሕመም ታሪክ አለ? ሀ. አዎ ለ. አይደለም

6. በቤተሰብዎ የደም ግፍት ታሪክ አለ? ሀ. አዎ ለ. አይደለም

7. እርስዎ የደም ግፊት አለቦት? ሀ. አዎ ለ. አይደለም

8. ሲጋራ አጭሰው ያቃሉ? ሀ. አዎ ለ. አይደለም ሐ. አቁሜያለሁ

9. ካጨሱስ ለምን ያህል ጊዜ? _____

10. ካጨሱ በቀን ምን ያህል ሲጋራ ያጨሳሉ? _____

11. አልኮል ይጠጣሉ? ሀ. አዎ ለ. አይደለም ሐ. አቁሜያለሁ

Part III a. English version of Androgen Deficiency in the Aging Male (ADAM) questionnaire about symptoms of low testosterone

Patients Card number_____

No	Questions	Yes	No
1	Do you have a decrease in libido (sex drive)?		
2	Do you have a lack of energy?		
3	Do you have a decrease in strength and/or endurance?		
4	Have you lost height?		
5	Have you noticed a decreased "enjoyment of life"		
6	Are you sad and/or grumpy?		
7	Are your erections less strong?		
8	Have you noticed a recent deterioration in your ability to play sports?		
9	Are you falling asleep after dinner?		
10	Has there been a recent deterioration in your work Performance?		

Part IIIb: Amharic version of ADAM questioner

ለሚከተሉት ጥያቄዎች ትይዩ አዎ ወይም አይደለም በሚሉት ረድፍ ላይ ትክክለኛ ምርጫዎን የ(X) ምልክት ያድርጉ፡፡

የታካሚዉ ካረድ ቁጥር----------

ተ.ቁ	ጥያቄዎች	አዎ	አይደለም
1	ያለወትሮዉ የወሲብ ፍላጎትዎ ቀንሷል?		
2	ያለወትሮዉ የአቅም ማነስ ችግር ገጥሞዎታል?		
3	ጥንካሬዎት ወይም ጽናትዎ ላይ መቀነስ አስተዉለዋል ?		
4	ቁመትዎ ከበፊቱ ቀንሷል?		
5	ያለወትሮው በሕይወትዎ ደስኛ ያለመሆን ሁኔታ አስተዉለዋል?		
6	የሀዘን እና የቁጣ ስሜት በተደጋጋሚ ይሰማዎታል?		
7	ብልትዎ ሲነሳ ጥንካሬ ያንሰዋል?		
8	የሰውነት እንቅስቃሴ የማድረግ ችሎታዎ የማሽቆልቆል ሁኔታ መኖሩን አስተዉለዋል?		
9	ከእራት በኋላ ወዲያው እንቅልፍ እንቅልፍ ይሎታል?		
10	ያለወትሮው በስራ አፈጻጸምዎ ላይ የማሽቆልቆል ሁኔታ አስተውለዋል?		

Annex IV: Data collection checklists

A Checklist to gather medical history and current medication from medical records

No	Parameters	Records	
1	Date of diagnosis of DM		
2	Current T2DM treatment		
3	Diabetic complications	Retinopathy 1. Yes 2. No Nephropathy 1. Yes 2. No Neuropathy 1. Yes 2. No	
4	Current medication of BP		

A Checklist to record anthropometric data and blood pressure

No	**Variables**	**Value**	Remark
1	Body weight		
2	Height		
3	BMI		
4	Waist circumference		
5	Blood pressure		

A Checklist to record laboratory findings

No	**Laboratory findings**	**Value**	**Remark**
1	TT		
2	LH		
3	FSH		
4	FBS		
5	TRIG		
6	HDL-C		
7	TC		
8	LDL-C		

Annex V. Standard operating procedure (SOP)

A. Sample collection

Fasting blood samples were taken from the anti-cubital vein of the arm by using syringes after proper antisepsis with alcohol and sterile cotton swabs in the morning before 10am. Then the blood from each participant were transferred to serum separator tube and allowed to stand for 30 minutes. Serum was separated by centrifugation at 1500 rpm. All the medical equipment used for blood collections were safe and sterile.

Procedure for serum separation

1. 5 ml whole blood was drawn into serum separator tube containing no anticoagulant.

2. It was kept in upright position at room temperature for 30-45 min to allow clotting.

3. It was centrifuged for 5 min at manufacturer's recommended speed 1500 rpm.

4. The serum was carefully aspirated at room temperature and pool into a centrifuge tube, taking care not to disturb the cell layer or transfer any cells. A clean pipette for each tube was used.

5. Serum will be inspected for turbidity. Turbid samples will be centrifuged and aspirated again to remove remaining insoluble matter.

6. Aliquot into cryovials and stored at –20 ℃. The cryovials were labeled with patient identification number.

B. Total testosterone test

Clinical relevance

Testosterone measurements are used in patient care for the diagnosis of hypogonadism in men and androgen excess in women with polycystic ovary syndrome being one of the conditions causing androgen excess. Research found that testosterone levels are associated with various diseases and conditions, such as metabolic syndrome, diabetes, cardiovascular disease, fractures, neurodegenerative disorder, and higher mortality in men with lower

testosterone levels. There is a need for population data to better define reference ranges and to further investigate associations between testosterone levels and chronic diseases.

Test principle and procedure of total testosterone test

Test principle - Competitive immunoassay with analyte liberation was used applied.

1st Incubation (9 minutes): 20 μL of the sample is incubated with a biotinylated monoclonal testosterone specific antibody and 2-bromoestradiol to release testosterone, with the amount of antibody binding sites subsequently occupied depending on the concentration of testosterone in the sample.

2nd Incubation (9 minutes): Streptavidin-coated micro-particles and a ruthenylated testosterone derivative are added to the reaction mixture and the complexes bind to the solid phase via biotin-streptavidin interactions.

Measurement method: Electrochemiluminscent

The reaction mixture is transferred to a measuring cell and the microparticles are magnetically captured onto the surface of an electrode; unbound sample is washed away before a chemiluminescent reaction is induced by applying a voltage to the electrode. Chemiluminescence is measured by a photomultiplier and the concentration of Testosterone within the sample is calculated using a calibration curve [53].

C. Test principle and procedure for LH test

Clinical relevance

Measurement of LH is used clinically to investigate dysfunctions within the hypothalamus-pituitary-gonad system. In conjunction with FSH, LH is also used to investigate congenital diseases with chromosome aberrations, polycystic ovaries, and to clarify causes of amenorrhea, menopausal syndrome, and suspected Leydig cell insufficiency

Test principle and procedure of LH test

1st Incubation (9 minutes): 20 μL of the sample is incubated with both a biotinylated, monoclonal LH-specific antibody and a ruthenylated, monoclonal LH-specific antibody to form a sandwich complex.

2nd Incubation (9 minutes): Streptavidin-coated microparticles are added to the reaction mixture and the complex binds to the solid phase via biotin-streptavidin interactions.

Measurement: The reaction mixture is transferred to a measuring cell and the microparticles are magnetically captured onto the surface of an electrode; unbound sample is washed away before a chemiluminescent reaction is induced by applying a voltage to the electrode. Chemiluminescence is measured by a photomultiplier and the concentration of LH within the sample is calculated using a calibration curve [54].

D. Test principle and procedure of FSH test

Sandwich principle. Total duration of assay: 18 minutes.

1st incubation: 24 µL of sample, a biotinylated monoclonal FSH-specific antibody, and a monoclonal FSH-specific antibody labeled with a ruthenium complexa) form a sandwich complex.

2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induce chemiluminescent emission which is measured by a photomultiplier. Results are determined via a calibration curve which is instrumentspecifically generated by 2-point calibration and a master curve provided via the cobas link [55].

E. Glucose oxidase test

Clinical relevance

Carbohydrates supply the body energy with glucose, which is the most important monosaccharide in blood, and it is an indispensable energy supplier for cellular function.

Measuring blood glucose is used for the diagnosis of carbohydrate metabolism disorders and monitoring of treatment in diabetes mellitus, neonatal hypoglycemia, idiopathic hypoglycemia, pharmic hypoglycemia and insulinoma [56].

Test Method: Glucose oxidase

Test principle

Glucose oxidase (GOD) converts the sample Glucose into gluconate. The Hydrogenperoxide (H2O2) produced in the reaction is degraded by peroxidase (POD) and gives a colored product Phenol and 4-Aminoantipyrine which is measurable using Trinder indicator reaction at 505 nm. The increase in absorbance correlates with the glucose concentration of the sample [56].

Specimen: Serum

Test procedure

	Blank	**Sample**
Reagent 1	240 µL	240 µL
Distilled water	3 µL	-
Sample	-	3 µL
Mix, incubate at 37 ℃ for 5 min., and read the blank absorbance, then add:		
Reagent 2	60 µL	60 µL
Mix thoroughly 37 ℃, and read the absorbance again 5-10 min. later.		
ΔA = [ΔA sample]- [ΔA blank]		

F. Lipid profile tests

i. Total cholesterol test

Clinical relevance

Cholesterol is a main component of cell membranes and lipoprotein and it is the precursor for steroid hormones and bile acids synthesizing. Cholesterol is transported in plasma by low-density lipoprotein. The level of the individual's total cholesterol is used in screening early atherosclerosis and monitoring the clinical effect of drugs or low-fat diet [57].

Test principle

Cholesterol is measured enzymatically in serum or plasma in a series of coupled reactions that hydrolyzes cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the

reaction byproducts, H_2O_2 is measured quantitatively in a peroxidase catalyzed reaction that produces a color. Absorbance is measured at 500 nm. The color intensity is proportional to cholesterol concentration. The reaction sequence is as follows [57] .

$$\text{Cholesteryl ester} + H_2O \xrightarrow{\textit{cholesteryl ester hydrolase}} \text{cholesterol} + \text{fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\textit{Cholesterol oxidase}} \text{cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\textit{Peroxidase}} \text{4-(p-benzoquinone-monoimino)-phenazone} + 4\ H_2$$

Test procedure

		Blank	**Sample**
Reagent		1000 µL	1000 µL
Distilled water		10 µL	-
Sample		-	10 µL
Mix thoroughly at 37℃, and read the absorbance 10 min later.			
ΔA = [ΔA sample]- [ΔA blank]			

ii. **Triglycerides test**

Clinical relevance

Triglycerides are the most abundant naturally lipids. It consists of three fatty acids and one glycerol, and is transported in plasma combing with apolipoproteins. Measurement of triglycerides is used for detecting early atherosclerotic risks, classing hyperlipoproteinemia and monitoring the clinical effect of drugs or low-fat diet. High triglyceride levels often lead to liver or kidneys disease, diabetes and pancreas disease [58].

Test principle

Triglycerides are measured enzymatically in serum or plasma using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase, and H_2O_2, one of the reaction products, is measured as described above for cholesterol. Absorbance is measured at 500 nm. The reaction sequence is as follows [58]:

Lipase

Triglycerides + $3H_2O$ ⟶ glycerol + fatty acids

Glycerokinase

Glycerol + ATP ⟶ glycerol-3-phosphate + ADP

Glycerophosphate oxidase

Glycerol-3-phosphate + O_2 ⟶ dihydroxyacetone phosphate + H_2O_2

Peroxidase

H_2O_2 + 4-aminophenazone + 4-chlorophenol ⟶ 4-(p-benzoquinone-monoimino)-phenazone + $2H_2O$ + HCl. [58]

Test procedure

		Blank	**Sample**
Reagent		1000 µL	1000 µL
Distilled water		10 µL	-
Sample		-	10 µL
Mix thoroughly at 37℃, and read the absorbance 10 min later.			
ΔA = [ΔA sample]- [ΔA blank]			

iii. HDL- C test

Clinical significance

HDL cholesterol is inversely related to the risk of developing coronary artery disease. A low HDL/LDL cholesterol ratio is directly related to the risk of developing coronary artery disease. High HDL cholesterol is associated with the "longevity" syndrome.

Test principle

The apoB containing lipoproteins in the specimen are reacted with a blocking reagent that renders them non-reactive with the enzymatic cholesterol reagent under conditions of the assay. The apoB containing lipoproteins are thus effectively excluded from the assay and only HDL-chol is detected under the assay conditions [59].

The reactions are as follows:

ApoB containing lipoproteins + α-cyclodextrin + Mg+2 + dextran SO_4 ⟶ soluble non-reactive complexes with apoB-containing lipoproteins

PEG-cholesteryl esterase

HDL-cholesteryl esters ⟶ HDL-unesterified cholesterol + fatty acid

PEG-cholesterol oxidase

Unesterified chol + O_2 ⟶ cholestenone + H_2O_2

H_2O_2 + 5-aminophenazone + N-ethyl-N-(3-methylphenyl)-N-succinyl ethylene diamine

Peroxidase + H_2O + H+ ⟶ qunoneimine dye + H_2O

Test procedure

	Blank	**Sample**
Reagent 1	900 µL	900 µL
Distilled water	12 µ	-
Sample	-	12 µL
Mix, incubate for 5 min. at 37℃, then add:		
Reagent 2	300 µL	300 µL
Mix thoroughly, incubate at 37℃ for 5 min., and then read the absorbance change value.		
ΔA = [ΔA sample]- [ΔA blank]		

iv. LDL-C test

Clinical significance

LDL-Cholesterol is directly related to the risk of developing coronary heart disease. A low HDL/LDL-Cholesterol ratio is directly related to the risk of developing coronary artery disease. Elevated LDL-Cholesterol is the primary target of cholesterol-lowering therapy [60].

Test principle

（1） HDL,VLDL, Chylomicrons ⟷ Cholestenone + H_2O_2

$2H_2O_2$ —*Catalase*→ $2H_2O + O_2$

（2） LDL ⟶ Cholestenone + H_2O_2

H_2O_2 + TOOS + 4-aminoantipyrin —*POD*→ Quinonimine

The System monitors the change in absorbance at 600 nm. This change in absorbance is directly proportional to the concentration of cholesterol in the sample and is used by the System to calculate and express the LDL-cholesterol concentration [60].

Test procedure

	Blank	**Sample**
Reagent 1	900 µL	900 µL
Distilled water	12 µ	-
Sample	-	12 µL
Mix, incubate for 5 min. at 37℃, then add:		
Reagent 2	300 µL	300 µL
Mix thoroughly, incubate at 37℃ for 5 min., and then read the absorbance change value.		
ΔA = [ΔA sample]- [ΔA blank]		

Declaration

I, the under signed, declare that this MSc thesis is my original work and it has not been presented for a degree in any other University. All sources of materials used for this thesis and institutions who gave support have been duly acknowledged.

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Sisay Teka Degechisa (BSc)

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   Signature: _____ Date: _____ Place: Addis Ababa Ethiopia

3. Kissi Mudi (MSc)

   Signature: _____ Date: _____ Place: Addis Ababa Ethiopia

4. Getahun Tarekegn (MD,MSc)

   Signature: _____ Date: _____ Place: Addis Ababa Ethiopia