Prevalence of low serum testosterone levels among men with type 2 diabetes mellitus attending two outpatient diabetes clinics in Durban, South Africa

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By

Dr Imran Mahomed Paruk
MBBCh (WITS), FCP (SA), CERT. ENDO (SA)

Supervisor
Professor Ayesha A Motala
MBChB (UKZN), MD (UKZN), FRCP (UK), FCP(SA)

Co-Supervisor
Dr. Fraser J Pirie
MBChB (UCT), MD (UKZN), FCP (SA)

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IMRAN MAHOMED PARUK

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LIST OF ABBREVIATIONS

ADAM - androgen deficiency of the ageing male

AMS - Ageing male's symptom questionnaire

ADAM - Androgen Deficiency in the Aging Male Questionnaire

ASA - American Society of Andrology

AUC - area under the curve

BMI - body mass index

CAG - trinucleotide, codon which codes for the amino-acid glutamine

CVD - cerebrovascular disease

DPP4 - dipeptidylpeptidase 4

EAU - European Association of Urology

EAA - European Association of Andrology

ELISA - enzyme linked immunosorbent assay

HbA1c - Haemoglobin A1c

HIV - human immunodeficiency virus

FT - free testosterone

FAI - free androgen index

HDL - high density lipoprotein

IALCH - Inkosi Albert Luthuli Central Hospital

IHD - ischaemic heart disease

ISA - International Society of Andrology

ISSAM - International Society for Study of the Aging Male

IQR – interquartile range

LCMS/MS - liquid chromatography tandem mass spectrometry

LDL - low density lipoprotein

LH - luteinizing hormone

LOH - late onset hypogonadism

LSFT - low serum calculated free-testosterone

LSTT - low serum total testosterone

NSFT- normal serum calculated free-testosterone

NSTT - normal serum total testosterone

PVD - peripheral vascular disease

RIA - radioimmunoassay

ROC - receiver operating characteristic

SHBG - sex hormone binding globulin

T2DM - type 2 diabetes mellitus

TT - total testosterone

WHO - World Health Organisation

WHR - waist to hip ratio

ABSTRACT

Background: Studies showing a high prevalence of low serum testosterone in men with type 2 diabetes mellitus (T2DM) are well documented but evidence from sub-Saharan Africa is scanty.

Aim: To determine the prevalence and associated risk factors of low serum testosterone and the prevalence of androgen deficiency symptoms in South African men with T2DM

Methods: A cross-sectional observational study was performed among men with T2DM attending two outpatient adult diabetes clinics in KwaZulu-Natal. Androgen deficiency symptoms were assessed using the Ageing Male's Symptom Scale (AMS) questionnaire and direct enquiry. Serum total testosterone (TT), sex-hormone binding globulin (SHBG), luteinising hormone (LH), HbA_{1c}, fructosamine, serum lipids were measured and free-testosterone (FT) was calculated. TT, SHBG and FT levels were measured in control subjects with no history of diabetes.

Results: The study included 148 men with T2DM (Study Group) and 50 control subjects (Control Group). The mean age of the control group was 43.9 ± 10.7 years and the mean BMI was 27.11 ± 4.2 kg/m^2. In the study group, the majority were African (Black) (58.7%); Indians (39.2%) and Whites (2.1%) constituted the remainder. Mean age was 57.5 ± 11.2 years; mean duration of diabetes 11.4 ± 8.9 years; mean HbA_{1c} was 8.6 ± 1.9%. Metabolic syndrome was found in 86.4% (n:127) of the Study group. Mean TT, SHBG, FT and median LH (IQR) in the Study group were within normal range (14.5 ± 5.8 nmol/l, 40.7 ± 20.3 nmol/l, 265.9 ± 90.4 pmol/l and 5.3 [3.8-7.3] IU/l, respectively). However, mean serum TT and FT was lower in the Study group than Control subjects (14.5 ± 5.8 vs. 18.8 ± 7.2 nmol/l, $p < 0.001$ and 265.9 ± 90.4 vs. 351.7 ± 127.3 pmol/l, $p<0.001$).

The prevalence of LSTT and LSFT was 35.8% and 16.2%, respectively. Prevalence of LSFT increased with age and higher body mass index (BMI) categories with the highest rate noted in >40 kg/m^2 BMI category (50%).

In multivariate analysis, LSFT was significantly associated with age [OR 1.05 (95% CI 1.02-1.218), p=0.043] and waist circumference (WC) [OR 1.033 (95% CI 0.999-1.068), p=0.059]. LSTT was associated with BMI only [OR 1.138 (95%CI 1.063-1.218), p<0.0001]. TT correlated inversely with BMI, WC and the number of metabolic syndrome criteria. FT correlated inversely with BMI, WC and WHR. For both FT and TT, no significant correlation was observed with HbA_{1c}.

The prevalence of androgen deficiency symptoms using AMS score was 74.5%. The prevalence of any androgen deficiency symptom on direct enquiry was 68.9%. The AMS score correlated poorly with LSTT or LSFT and was not superior to direct enquiry.

Conclusion: In this group of predominantly African and Indian men with T2DM from KwaZulu-Natal, there was a high prevalence of LSTT and LSFT. Serum TT and FT was lower in men with T2DM compared to control subjects. Waist circumference was a significant risk factor associated with LSFT while LSTT was associated with higher BMI and older age. There was a high prevalence of androgen deficiency symptoms using both the AMS score and on direct enquiry. The AMS score was a poor predictor of low testosterone and was not superior to direct enquiry. More research is required locally and from other sub-Saharan African countries before routine screening can be recommended.

CHAPTER 1: INTRODUCTION AND BACKGROUND

The global prevalence of type 2 diabetes mellitus (T2DM) is escalating at an alarming pace with the International Diabetes Federation projecting that 642 million people will be affected by 2040 (1). In Africa alone, there will be more than a doubling in the total number of people living with diabetes between 2015 and 2040. This trend appears to mirror that being witnessed worldwide with respect to the global obesity epidemic. South Africa is not immune to this trend and is estimated to have among the highest rates of overweight in sub-Saharan Africa (SSA). The reported prevalence of obesity among South African women is 42%, while the prevalence of both overweight and obesity is 69.3%. Among men, the prevalence of overweight or obesity is reportedly 39%, with 14% being obese (2). Therefore South Africa ranks among the top obese nations of the world, with the associated negative impact on diabetes prevalence anticipated in the near future (3).

Male androgen deficiency is an international health-related issue that has received increased attention over the past two decades and has generated a number of trials in men with diabetes. The prevalence of androgen deficiency in middle-aged and older men was found to be between 6 and 12% in the Massachusetts Male Aging Study (4). Testosterone deficiency has been associated with a plethora of disorders including T2DM, obesity, cardiovascular disease and the metabolic syndrome. The reported prevalence of low testosterone levels among men with T2DM is high and ranges between 30% and 50% (5,6). The significance of this association with T2DM has long been debated with many experts arguing that low total testosterone levels are a consequence of a low sex-hormone binding globulin (SHBG) level which in turn is related to insulin resistance. However, this argument is unlikely to completely explain the relationship since the association with T2DM has also been

demonstrated with free testosterone levels. The aetiology for this association remains uncertain, but it has been postulated that a hypothalamic or pituitary cause is likely, given the predominant finding of normal or low luteinizing hormone (LH) levels with low testosterone levels (6). International guidelines advocate screening with a total testosterone assay, but recommend measuring SHBG and calculating free testosterone levels in patients with total testosterone levels near the lower limit of the normal range or those in whom alterations of SHBG are suspected (5).

Defining low testosterone levels

The finding of a low testosterone level alone does not imply hypogonadism or signify a clinical disease entity. Rather, a diagnosis of hypogonadism requires an unequivocally low testosterone level in conjunction with typical signs or symptoms of hypogonadism (6). Therefore it would be important to elicit symptoms of androgen deficiency from men with T2DM. Such symptoms include loss of libido, loss of energy, erectile dysfunction, hot flushes and reduced stamina. In this regard, a number of screening tools in the form of self-administered questionnaires have been developed to assess for the symptoms of androgen deficiency and its severity. These include the Ageing Male's Symptom Scale (AMS), Massachusetts Male Aging Study (MMAS) questionnaire and Androgen Deficiency in the Ageing Male Questionnaire (ADAM) (7). Although the benefits of employing these questionnaires in diabetic men may seem logical, none of the available questionnaires are sensitive or specific enough to be able to make a conclusive diagnosis of hypogonadism. Also, there is currently insufficient proof to support their routine use in clinical practice. Furthermore, none of these screening questionnaires have been validated in African men. Despite this lack of information, many physicians continue to use these questionnaires in the absence of an evidence based alternative screening tool

Furthermore, the actual laboratory definition of a low testosterone level is itself controversial for a number of reasons and therefore presents a dilemma to those wanting to easily diagnose the condition. At the core of the argument is the absence of any classical sign or symptom of testosterone deficiency or any definitive injurious clinical consequences associated with a particular lower limit of serum testosterone. Moreover, there are notable variations in the normal reference ranges of serum testosterone level between laboratories. Consequently, there is no concordance with respect to the definition of a low testosterone level among healthcare practitioners as well as between laboratories. Even more controversial is the derivation of the currently accepted “normal” assay range for serum total testosterone used by laboratories which are generally obtained using convenience sampling, usually from small groups which may have an over-representation of older men. It is therefore questionable whether this range is representative of the sexual and physical health in the general population of men. Ideally, normal ranges should be age and population specific with individual reference ranges derived from healthy controls for each demographic region, though this is unlikely to occur due to cost constraints.

In 2009, a Consensus Statement on the treatment and monitoring of late onset hypogonadism in males was issued jointly by the International Society of Andrology (ISA), the International Society for Study of the Aging Male (ISSAM), the European Association of Urology (EAU), the European Association of Andrology (EAA) and the American Society of Andrology (ASA)(8). This consensus recommended that total testosterone (TT) levels above 12nmol/l do not require treatment, and levels below 8nmol/l (with symptoms) may require testosterone replacement therapy. For levels between 8-12nmol/l, the consensus statement recommended repeating TT and calculating free-testosterone (FT). A year later, the Endocrine Society guideline

recommended that total low testosterone be defined using the lower limit of normal range for healthy young men established in the laboratory performing the assay (4). However, there are no generally accepted lower limits of normal FT for the diagnosis of hypogonadism. If the evidence for an association between TT and typical symptoms of androgen deficiency is weak, then the relationship is even weaker with FT. Since the measurement of TT is clearly more economical, it remains the test recommended by most authorities for the general population.

Circulating total and free testosterone

Testosterone is the male hormone synthesized primarily by the Leydig cells in response to stimulation of the testes by luteinizing hormone. Once secreted into the circulation, it may exist either in a free state or bound to plasma proteins. Testosterone may circulate bound to SHBG (44%), cortisol binding globulin (4%) or albumin (50%) and about 2-3% actually remains unbound. Testosterone is loosely bound to albumin and the term “bioavailable testosterone” is used to describe the sum of free and albumin-bound testosterone. Since testosterone is to a large degree bound to SHBG, the measurement of TT is greatly influenced by changes in SHBG level. Conditions that increase serum SHBG and hence TT include ageing, liver cirrhosis, hepatitis, thyrotoxicosis, oestrogen therapy, anticonvulsant use and human immunodeficiency virus (HIV) infection. In contrast, serum SHBG may decline with obesity, T2DM, nephrotic syndrome, hypothyroidism, acromegaly or with the use of glucocorticoids and progestins.

Laboratory measurement of testosterone

Serum TT remains the test recommended by most endocrine societies and laboratories due to its wider availability, cost efficiency and greater validation from

research data when compared to FT. Results of laboratory measurement of TT can be significantly influenced by the type of assay method employed. The most accurate measurement of testosterone status remains equilibrium dialysis, but this is labour intensive and costly. Older assay methods employed radioimmunoassay (RIA) which is based on competitive binding of testosterone in serum to a testosterone specific antibody after pre-exposure to a fixed amount of radioactive labelled tracer. Most laboratories employ the ELISA (enzyme linked immunosorbent assay) technique which is based on the same principle of competitive binding of testosterone used in the RIA with the exception that a non-radioactive tracer and a fixed amount of testosterone antibodies are employed with the ELISA method. More accurate assay methods include liquid chromatography tandem mass spectrometry (LCMS/MS) which are more expensive and often used in research settings but are increasingly finding their place in clinical laboratories as the cost of performing these tests decline.

Low serum testosterone and ageing

There is considerable epidemiologic evidence demonstrating a decline in testosterone levels with ageing. In the Baltimore Longitudinal Study of Aging (BLSA) cohort made up of 3,565 middle-class, mostly Caucasian men in the United States, the incidence of LSTT increased from 20% of men aged over 60 years, 30% over 70 years, to 50% over 80 years-of-age (9). The Massachusetts Male Aging Study (MMAS) showed a similar high prevalence among a cohort of 415 healthy men and 1294 men with chronic disease aged between 40 and 70 years (10). Age related decline in testosterone levels, often referred to as late onset hypogonadism (LOH) is also referred to as andropause or androgen deficiency of the ageing male (ADAM). LOH is defined as a clinical and biochemical syndrome associated with advancing age and characterised by symptoms and a deficiency in serum testosterone levels.

The effects of LOH may include erectile dysfunction, loss of libido, loss of muscle mass and increased abdominal adiposity. LOH has also been associated with metabolic syndrome, cardiovascular disease and mortality.

Relationship between low serum testosterone and type 2 diabetes mellitus

The reported prevalence of low testosterone levels among male subjects with T2DM is high and ranges between 30% and 50% (5,6). This evidence comes mainly from small population based studies but is also supported by at least two large meta-analyses (11,12). Using multiple regression models, after adjusting for age and body mass index (BMI), T2DM was still associated with lower total testosterone (TT) levels; there seems to be an inverse relationship between TT and T2DM (11) . Conversely, longitudinal studies have also shown that men with higher testosterone levels have a lower risk of developing type 2 diabetes (11,12). A single study from South Africa showed a high prevalence (50%) of low serum testosterone among men with diabetes attending a tertiary diabetes clinic at an academic centre in Pretoria. Despite the available evidence, current international diabetes guidelines do not advocate routine screening for testosterone levels among male patients with diabetes. Longitudinal studies have also reported lower TT levels among men with metabolic syndrome compared to controls. More prospective studies are required to determine the true relationship between these two syndromes.

Aetiology of low serum testosterone in men with diabetes

There is no clear explanation for the recognised association but a number of theories have been proposed which in large part are linked to obesity. For example, adipose tissue related elaboration of inflammatory cytokines (adipokines) may have a suppressive effect on hypothalamic function leading to a state of functional

hypogonadotropic hypogonadism (13). Furthermore, obesity *per se* is associated with lower SHBG levels and this may impact on the measurement of TT. It has also been postulated that LST in T2DM is related to chronic illness similar to that observed with other chronic diseases such as HIV and chronic kidney disease (13).

Testosterone and glucose metabolism

Although some studies have shown an association between low testosterone levels and glucose metabolism including a higher HbA1c, it is unclear if therapy with testosterone will yield any significant gains in glycaemic control or insulin sensitivity. Studies in which replacement therapy has been used for testosterone deficiency have thus far shown conflicting results with some showing no changes in metabolic parameters such as glucose, lipids and HbA1c (15). Further large-scale studies are needed to investigate the potential for long term benefits regarding the treatment of diabetic patients with testosterone deficiency.

Influence of Ethnicity on testosterone levels

There are few studies that have shown ethnic differences in the levels of serum testosterone. A study from the United States showed that whilst TT, estradiol and SHBG did not differ between black and white men, after adjustment for age, black men had a modest but significant 2.5 to 4.9% higher FT level than white men (14). Suggested reasons for these findings include variances in steroid metabolism, geographic or environmental factors, disparity in body composition and genetic differences such as polymorphisms in the androgen receptor gene and length of the CAG repeat chain (13).

Data from sub-Saharan Africa

There is a dearth of information on the prevalence of androgen deficiency symptoms and low serum testosterone levels in men with T2DM, from sub-Saharan Africa (SSA). To date published data is only available from only three countries, including Nigeria, Ghana and South Africa (16–20). These studies have shown high prevalence of low serum testosterone levels ranging from 29.5-50%. Available data from South Africa is limited to a single study from an academic centre in Pretoria (16). Furthermore, only a few studies have actually investigated the risk factors that are associated with low testosterone.

Rationale for the study

There is scanty information from South Africa regarding the prevalence of low serum testosterone levels among male subjects with T2DM. This study was therefore designed to determine the prevalence and associated risk factors of low serum testosterone levels in men with T2DM in KwaZulu-Natal.

CHAPTER 2: AIMS AND OBJECTIVES

2.1 Study Aim

To determine the prevalence and associated risk factors of low serum testosterone levels in South African men with type 2 diabetes mellitus attending two outpatient diabetes clinics in Durban.

2.2 Study Objectives

Primary Objective:

To determine the prevalence of a low serum testosterone level among male patients with type 2 diabetes mellitus.

Secondary Objective:

a) To determine the prevalence of symptoms of androgen deficiency

b) To determine the serum luteinizing hormone level among patients with low serum testosterone levels

c) To determine the association between low serum testosterone and metabolic parameters

2.2 Hypothesis

The hypothesis is that the prevalence of testosterone deficiency in South African men with type 2 diabetes is high and that most patients with testosterone deficiency remain undiagnosed.

CHAPTER 3: RESEARCH DESIGN AND METHODS

3.1 STUDY DESIGN

This was a cross-sectional observational study.

3.2. STUDY POPULATION

3.2.1 Study Subjects

All male patients attending the adult diabetes clinic at Inkosi Albert Luthuli Central Hospital (IALCH) or the medical outpatient diabetes clinic at Prince Mshiyeni Memorial Hospital (PMMH) were invited to participate in this study. The nature of the study and its aims were explained to the patients and participation was completely voluntary. Signed informed consent was obtained from all patients before entry into the study.

Inclusion criteria:

- Adult male patients aged over 18 years with a diagnosis of T2DM according to the World Health Organisation (WHO) definition
- Signed informed consent

Exclusion criteria:

- Unwillingness to participate
- Diabetes classified other than type 2 diabetes
- Disorder of sexual development

3.2.2 Control Subjects

Source of control subjects:

Control subjects included healthy adult men employed at either IALCH or PMMH, Durban or relatives of patients attending the diabetes clinic. Potential for bias was minimised by excluding patients with a history of glucose intolerance, low testosterone (or hypogonadism) or disorder of sexual development.

Inclusion criteria:

- Age ≥18 years
- Signed, informed consent

Exclusion criteria:

- Unwillingness to participate
- Known to have testosterone deficiency (hypogonadism) or disorder of sexual development
- Any past or present abnormality of glucose tolerance as ascertained from History

Sample Size:

The sample size was based on the prevalence of low serum testosterone levels in men. Assuming a ratio of unmatched cases to controls of 1:1, to detect a 20% difference between diabetic and non-diabetic men at 95% confidence interval and 80% power, the estimated sample size was 49 participants in each arm; total sample size of 98 participants.

3.3 STUDY METHODS

For all study subjects, information was collected at study entry and is outlined in the data sheet (Appendix 1) and included the following:

3.3.1 History

3.3.2 Clinical examination

3.3.3 Laboratory tests

3.3.1 History

A detailed history was obtained from all subjects at entry into the study. This was obtained at the first interview via direct patient enquiry and patient folder review, and included the following:

(a) Demographic details

- age
- ethnic group

(b) Diabetes

- date of diagnosis
- duration (number of years)
- microvascular complications (type)
- macrovascular complications (type)

(c) Past medical history

- testosterone deficiency or hypogonadism

- current or past treatment with testosterone of testosterone ester
- current or past exposure to anabolic steroids
- radiotherapy, chemotherapy, testicular torsion
- orchidopexy or orchidectomy
- previous orchitis eg mumps or TB
- trauma to testes eg crush injury to pelvis and/or gonads
- head trauma, neurosurgery to pituitary fossa

(d) Current Drug therapy (including drug doses for antidiabetic therapy)

(e) Smoking history

(f) Enquiry of symptoms

- change in growth of facial hair (beard growth)
- loss of libido
- erectile dysfunction

(g) Patient questionnaire: Ageing Male's Symptom (AMS) Questionnaire (7) in English and Zulu languages (Appendix 2 and Appendix 3)

- Symptoms of androgen deficiency were assessed in all patients with the aid of the AMS questionnaire
- Details of the patients' responses to all 17 questions in the Ageing Male's Symptom (AMS) questionnaire were captured
- The AMS questionnaire includes 3 sub-categories consisting of a defined set of items
- Each question is rated on a scale of 1 to 5 points

3.3.2 Clinical Information

Clinical examination was performed on all patients and recorded on the day of serum testosterone sample collection. The following parameters were documented:

- Weight
- Height
- Hip circumference
- Waist circumference
- Systolic and diastolic blood pressure

3.3.3 Laboratory Evaluation

The following laboratory tests were performed on all patients included in the study:

- serum total testosterone (TT)
- serum sex-hormone binding globulin (SHBG)
- free testosterone (FT) calculated, free androgen index (FAI) calculated
- luteinizing hormone (LH)
- gamma glutamyl transferase (GGT)
- alkaline phosphatase (ALP), albumin, total protein
- alanine transaminase (ALT), aspartate transaminase (AST)
- total triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol
- glycated haemoglobin (HbA1c) and serum fructosamine
- serum urea, electrolytes and creatinine
- full blood count (FBC)

Sampling procedure

Venous blood samples were collected from patients between 08h00 and 10h00.

Venous blood was drawn from a forearm vein and collected into the following tubes:

- 1 x 5ml Sodium fluoride containing tubes: plasma glucose
- 2 x 10ml Plain tubes: serum lipids, serum urea, electrolytes and creatinine, serum liver function tests (LFT), fructosamine, total testosterone, SHBG, luteinising hormone
- 2 x 5ml EDTA tubes: FBC and HbA1c

All specimens for LH, SHBG, total and free testosterone were centrifuged and the plasma transferred to Eppendorf tubes for storage in a freezer at - 70^0C in the Department of Internal Medicine laboratory at Nelson R Mandela School of Medicine. All stored specimens were analysed at Lancet laboratories in Durban as a single batch.

Assay Methods

Test:	**Method**	**Platform**
HbA1c	HPLC	TOSOH G8
Fructosamine	Calorimetry	Siemens Dimensions
Lipid profile		Siemens Advia 1800
Total testosterone	Chemiluminescence	Abbot architect
Luteinizing hormone	Chemiluminescence	Abbott architect
SHBG	Chemiluminescence	Abbott architect
Free-testosterone	Calculated Vermeulen formula *	
Free androgen index	Calculated Index[$]	

* Vermeulen formula: FT 5([T]2(N3 [FT]))/(Kt{SHBG 2 [T] 1 N[FT]}) (Eq IV), where Kt is the association constant of SHBG for T, and N 5 KaCa 1 1

[$] FAI formula: total testosterone (nmol/ L)) x 100 /sex hormone-binding globulin (SHBG) (nmol/ L)

HPLC: High performance liquid chromatography

SHBG: sex hormone binding globulin

3.4 DEFINITIONS

3.4.1 Low serum testosterone

There is no accepted global definition for low serum total testosterone (LSTT). In this study LSTT was defined as a measured serum total-testosterone level below 12.0 nmol/l (21). However, other levels of LSTT will also be determined:

- LSTT < 8 nmol/l (21)
- Borderline TT includes men with a TT that ranges between 8-12 nmol/l.

Low serum free-testosterone (LSFT) was defined as a calculated free-testosterone level below the reference range for the laboratory (< 180 pmol/l) [lower limit of laboratory assay with a reference range of 180-739 pmol/l].

The Endocrine Society Clinical Practice Guideline definition of low testosterone includes patients with unequivocal LSTT and a LSFT in men with TT concentrations near the lower limit of the normal range [i.e. LSTT < 8 nmol/l and patients with TT between 8-12 nmol/l that have a FT<180 pmol/l (lower limit of laboratory assay with a range of 180-739 pmol/l)] (5).

3.4.2 Androgen deficiency symptoms

Patients with an AMS questionnaire score of ≥ 27 were defined as being consistent with LST and androgen deficiency. The classification of androgen deficiency symptoms (7) was based on the total score for each patient, as shown below:

- < 26: no symptoms consistent with LST
- 27-36: mild symptoms consistent with LST
- 37-49: moderate symptoms consistent with LST

- > 50: severe symptoms consistent with LST

3.4.3 Hypogonadism

Hypogonadism was defined using serum free-testosterone and serum total-testosterone criteria. A diagnosis of hypogonadism was considered when low testosterone (total or free) has been documented in conjunction with symptoms of androgen deficiency (8).

Using free-testosterone as the criterion, hypogonadism was defined as a low serum free-testosterone <180 pmol/l and a positive AMS score of 27 or more.

Using total-testosterone as the criterion, hypogonadism was defined as a low serum total-testosterone < 12nmol/l (21) and a positive AMS score of 27 or more. Overt hypogonadism was defined as a total-testosterone <8 nmol/l and a positive AMS score of 27 or more. Possible hypogonadism was defined as a total-testosterone between 8 and 12 nmol/l and a positive AMS score of 27 or more.

3.4.4 Classification of Luteinizing Hormone (LH) levels

The laboratory reference range for LH levels vary between 3.8 mIU/L to 7.3mIU/L and values within this range were classified as normal. LH levels below 3.8 mIU/L were classified as low and values greater than 7.3mIU/L were classified as high.

3.4.5 Metabolic Syndrome

Metabolic syndrome was defined by the criteria of the Joint Interim statement (22). The presence of any 3 of 5 risk factors constitutes a diagnosis of metabolic syndrome:

- Elevated waist circumference (For Indian men ≥ 90cm; Indian women ≥ 80cm; African men ≥ 94cm; African women ≥ 80cm)
- Elevated triglycerides ≥ 1.7mmol/l (drug treatment for elevated triglycerides is an alternate indicator)
- Reduced HDL-C <1.0 mmol/L in males; females <1.3 mmol/L (drug treatment for reduced HDL-C is an alternate Indicator)
- Elevated blood pressure; systolic ≥ 130 mmHg and/or diastolic ≥ 85mmHg (anti-hypertensive drug treatment in a patient with a history of hypertension is an alternate indicator)
- Elevated fasting blood glucose ≥ 5.6mmol/l (drug treatment of elevated glucose is an alternate indicator)

3.5. Ethics statement

The study was approved by the University of KwaZulu-Natal Biomedical Research Ethics Committee (BREC) and KwaZulu-Natal Department of Health (KZN DOH). BREC reference number: BF 225/13. Permission to conduct research was also obtained from IALCH and PMMH. Consent was obtained from each patient as required by hospital management, KwaZulu-Natal Department of Health, University of KwaZulu-Natal Biomedical Research Ethics Committee. All data was anonymized.

3.6 Statistical Analysis

The IBM Statistics Package for the Social Sciences (SPSS) version 23 was used to analyse the data. Descriptive analysis of the data was performed (means, standard deviations, ranges, frequencies and percentages). Independent t tests were used to compare continuous normal variables between binary groups. Pearson's chi square tests were used to compare categorical variables between groups. Missing continuous data was replaced by the series mean.

Univariate logistic regression analysis was used to screen all risk factors at the 0.1 level of significance for inclusion into a multiple logistic regression model. Once predictors were chosen, they were entered into a backwards stepwise model with entry and exit probabilities set at 0.05 and 0.1 respectively. The final model is shown with odds ratio (OR) and 95% CI (confidence interval). Receiver operating characteristic (ROC) curves were used to calculate AUC (area under curve) and if appropriate, sensitivity and specificity of the cut points.

CHAPTERS 4 - 12: RESULTS

CHAPTER 4: CHARACTERISTICS OF THE TOTAL STUDY POPULATION

The total study population included 148 male subjects with type 2 diabetes mellitus (Study Group) and 50 control subjects (Control Group)

4.1 Study Group

Of 157 patients who consented to participate in the study, 97 men were recruited at Inkosi Albert Luthuli Hospital and 60 men were recruited at Prince Mshiyeni Memorial hospital. A total of nine patients were excluded because they were known to have hypothalamic or pituitary disease. The study group for this analysis included 148 male patients with type 2 diabetes mellitus.

4.1.1 Demographic and clinical characteristics

Table 1 and Figure 1 shows the characteristics of the study group. The mean age of the study group was 57.5 ± 11.2 years. The majority were African (black) (58.7%) (n=87) followed by Indian patients (39.2%) (n=58); white patients were in the minority and constituted only 2.1% (n=3) of the study population. The mean duration of diabetes was 11.4 ± 8.9 years; almost half (47%) had diabetes duration > 10 years. The overall prevalence of current cigarette smokers and ex-smokers was 37.2% (n=55), with a median (IQR) duration of smoking of 9 pack-years (0.5-60) (Table 1).

The prevalence of known diabetes related complications among study subjects is shown in Figure 1. Microvascular complications including retinopathy,

microalbuminuria and proteinuria were documented in 23.6%, 22.9% and 18.2% of patients, respectively. A past history of any known macrovascular disease was present in 22.2% of patients with the most common being ischaemic heart disease (IHD) (17.6%); peripheral vascular disease (PVD) and cerebrovascular disease (CVD) were recorded in 7.4% and 4.1% of subjects, respectively.

Mean systolic and diastolic blood pressure was 136 ± 16.5 and 77.3 ± 10.0 mmHg, respectively. Anthropometric measures are shown in Table 1. The mean height and weight were 170 ± 0.06 cm and 85.9 ± 17.8 kg, respectively; mean BMI was 29.8 ± 6.0 kg/m^2. The mean waist circumference was 103.3 ± 12.9 cm and mean waist to hip ratio (WHR) was 0.97 ± 0.07. The majority of the patients were overweight or obese (81.8%) and only 18.2% had a BMI below 25 kg/m^2 (Figure 2). Obesity was noted in 41.3% of patients with 18.2% being classed as morbid obesity. The prevalence of metabolic syndrome was 85.8%. All five criteria were present in 14% of patients and only one patient was found to have no criteria of metabolic syndrome.

4.1.2 Medication history in the study group

Medication history in the Study group is shown in Table 2. Regarding anti-hyperglycaemic therapy, 42.9% were on oral agents, 32.9% on combination oral and insulin therapy and 24.2% on insulin alone. Among those using oral agents alone or in combination with insulin, the majority of patients were treated with metformin (71.4%) (n=100); 31.4% (n=44) were on a sulphonylurea and 1% were treated with a dipeptidylpeptidase-4 (DPP4) inhibitor. The median daily metformin dose was 2000mg. Among sulphonylurea users, glibenclamide was used in 9.3% (n=13) and gliclazide in 21.5% (n=31). The median daily glibenclamide and gliclazide dose was

12.5mg and 160mg, respectively. Over half the patients (55.7%) were on insulin therapy, either alone or in combination with oral agents. The median daily insulin dose prescribed was 58 (0-63) IU.

Regarding non-glycaemic therapy, 65.7% (n=92) were on statin therapy and 3.6% (n=5) on fibrates. The majority (83.4%) were on anti-hypertensive agents and two-thirds on aspirin therapy. The most commonly prescribed anti-hypertensives were the angiotensin converting enzyme inhibitors (ACE-I) and angiotensin receptor antagonists (ARB) (68.6%). Among anti-hypertensive therapy users, only 18.8% of patients were taking a single agent while the majority were on multiple agents (81.2%). Two anti-hypertensive agents were prescribed in 37.6% (n=44) of patients; three agents in 29.9% (n=35); four agents in 10.3% (n=12) and five agents were used in 3.4% (n=4) of those on therapy.

4.1.3 Laboratory test results in the Study Group

The laboratory results for the Study group are shown in Table 3. The mean HbA_{1c} was 8.6 ± 1.9 % and mean serum fructosamine, 317.5 ± 71.7 μmol/l. Mean serum total cholesterol was 4.2 ± 1.3 mmol/l, mean serum HDL cholesterol 1.1 ± 0.3 mmol/l, mean serum LDL cholesterol 2.5 ± 1.41 mmol/l and median serum total triglycerides 1.7 (1.1-2.7) mmol/l. The hormonal profile showed a mean TT of 14.5 ± 5.8 nmol/l, mean SHBG level of 40.7 ± 20.3 nmol/l, FAI of 40.4 ± 17, FT of 265.9 ± 90.4 pmol/l. The median LH level was 5.3 (3.8-7.3) IU/l.

4.2 Control Group

Table 4 shows the characteristics of the control group. The mean age of the control group was 43.9 ± 10.7 years and the mean BMI was 27.11 ± 4.2 kg/m^2. Mean systolic and diastolic blood pressure were 130.86 ± 21.6 mmHg and 76.39 ± 8.6 mmHg respectively.

4.3 Clinical characteristics in the Study Group vs. Control Group

When compared with the Control group, patients with T2DM (Study group) were significantly older (57.7 ± 11.1 vs 43.9 ± 10.7 years; p<0.001), had a higher BMI (29.8 ± 6 vs 27.1 ± 4.2 kg/m2; p=0.01) but had similar systolic and diastolic blood pressures (Table 4).

Table 1: Characteristics of the study group (n:148)

Study Group	
Age (years)	57.5 ± 11.2
Ethnicity	
African	87 (59%)
Indian	58 (39%)
White	3 (2%)
Diabetes duration (years)	11.4 ± 8.9
Smoking (current or Ex)	55 (37%)*
Systolic pressure (mmHg)	136 ± 16.5
Diastolic pressure (mmHg)	77.3 ± 10.0
Height (cm)	170.0 ± 0.06
Weight (kg)	85.9 ± 17.8
BMI (kg/m^2)	29.8 ± 6.0
Waist (cm)	103.3 ± 12.9
Waist:hip (WHR)	0.97 ± 0.07

Results expressed as Mean ± SD or (n) % except where indicated.
* Median pack/years of smoking (IQR): 9 (0.5-60) years

Table 2: Medication history in the study group (n:140)

Medication	N	%	Median dose (IQR)
Metformin (mg)	100	71.4	1000 (1000-2000)
Sulphonylurea	44	31.4	
Gliclazide (mg)	31	21.5	160 (40-320)
Glibenclamide (mg)	13	9.3	12.5 (5-20)
DPP4i	2	1.4	
Insulin (IU)	78	55.7	58 (0-63)
Statin	92	65.7	
Fibrate	5	3.6	
Anti-hypertensive	117	83.6	
ACEI/ARB	96	68.6	
Diuretic	83	59.3	
Amlodipine	58	41.4	
Doxazosin	19	13.6	
Beta-blocker	30	21.4	
Aspirin	93	66.4	

DPP4i: dipeptidyl peptidase inhibitor; ACEI: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker

Table 3: Laboratory results in the study group

Metabolic profile*	
HbA_{1c} (%)	8.6 ± 1.9
Serum fructosamine (μmol/l)	317.5 ± 71.7
Serum lipids (mmol/l)	
Total cholesterol	4.2 ± 1.3
Total triglycerides	1.7 (1.1-2.7)
HDL cholesterol	1.1 ± 0.3
LDL cholesterol	2.5 ± 1.41
Hormonal profile (n:148)	
Serum total testosterone (nmol/l)_	14.5 ± 5.8
Serum SHBG (nmol/l)	40.7 ± 20.3
Free androgen index	40.4 ± 17.0
Serum free testosterone (pmol/l)	265.9 ± 90.4
Serum LH (mIU/L)	5.3 (3.8-7.3)

Mean ± SD or median (IQR)
*HbA_{1c} (n:145); fructosamine (n:88); lipid profile (n:128)
HbA_{1c}: Haemoglobin A1c; HDL: high density lipoprotein; LDL: low density lipoprotein; SHBG: sex hormone binding globulin; LH: luteinizing hormone

Table 4: Clinical and biochemical characteristics in the study group (n:148) compared to the control group (n:50)

	Study group (n:148)	Control group (n:50)	P
Clinical characteristics			
Age (years)	57.7 ± 11.1	43.9 ± 10.7	<0.001
BMI (kg/m^2)	29.82 ± 6.0	27.11 ± 4.2	0.011
Systolic pressure (mmHg)	135.78 ± 16.5	130.86 ± 21.6	0.134
Diastolic pressure (mmHg)	77.30 ± 10.0	76.39 ± 8.6	0.613
Biochemical results (serum)			
Total testosterone (nmol/l)	14.5 ± 5.8	18.83 ± 7.2	<0.001
SHBG (nmol/l)	40.7 ± 20.3	42.0 ± 20.0	0.685
Free androgen Index	40.4 ± 17.0	49.6 ± 19.5	0.002
Free testosterone (pmol/l)	265.9 ± 90.4	351.7 ± 127.3	<0.001
LH (IU/L)	5.8 ± 3.1	4.5 ± 2.3	0.006

Results expressed as Mean ± SD
BMI: body mass index; SHBG: sex hormone binding globulin; LH: luteinising hormone

Figure 1: Prevalence of diabetes-related complications in the study group (n:148)

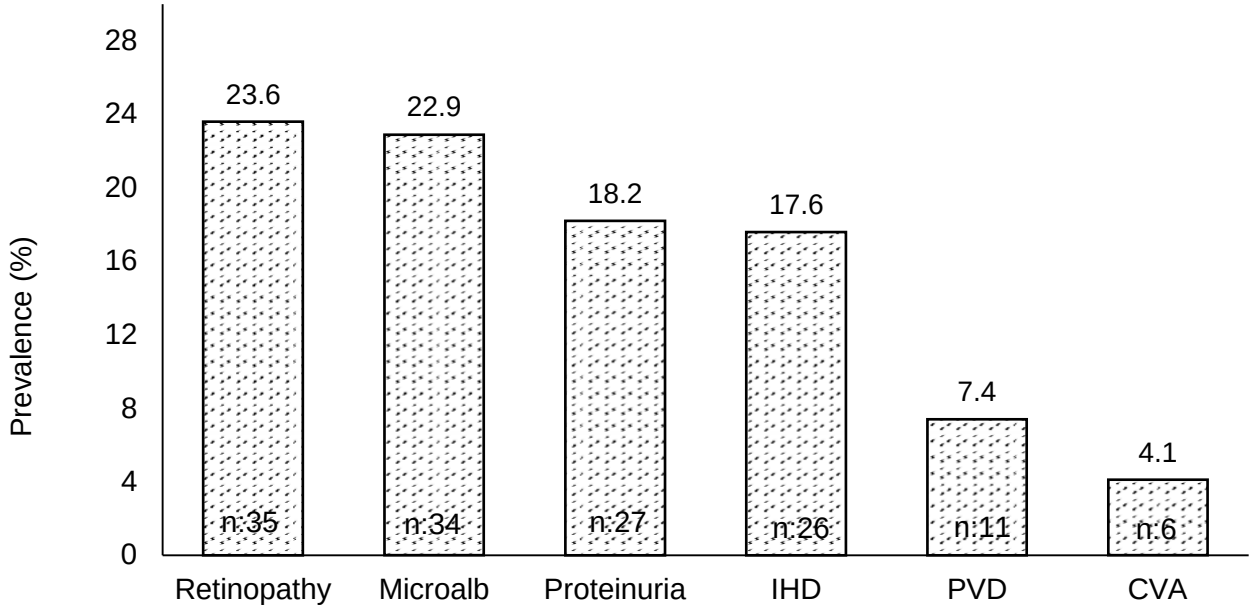
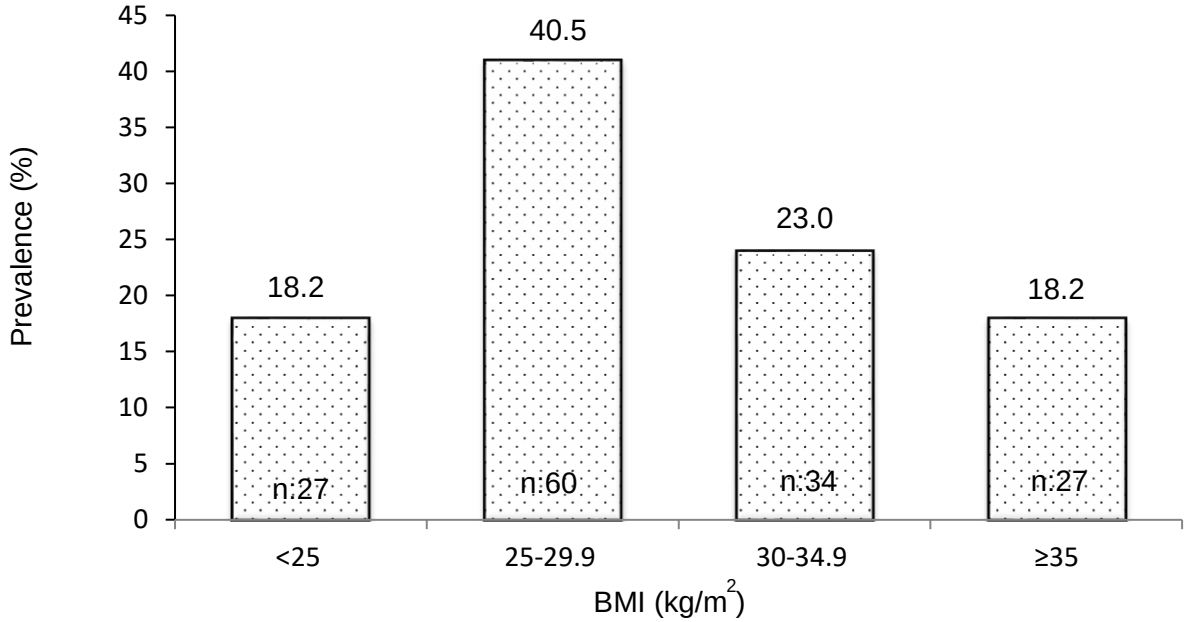


Figure 2: Body mass index (BMI) distribution in the study group (n:148)



CHAPTER 5: SERUM TOTAL TESTOSTERONE, FREE TESTOSTERONE, SEX-HORMONE BINDING GLOBULIN AND FREE ANDROGEN INDEX IN THE STUDY POPULATION

5.1 Serum free-testosterone (FT), total testosterone (TT) and sex-hormone binding globulin (SHBG) by age group and BMI category in the Study Group

Mean serum FT, TT and SHBG in the Study group have been discussed in the previous chapter (Table 3). Figure 3 shows the serum FT levels across age groups. Mean serum FT levels were lower with increasing age; the highest free testosterone levels were observed in the youngest group (< 40 years) and the lowest mean FT levels were noted in the oldest group (≥70 years). A significant difference ($p<0.05$) was found between the following age groups: <40 yr vs. 40-49 yr; 50-59 yr vs. 60-69 yr and 60-69 yr vs. ≥70 yr.

Figure 4 shows the mean TT, SHBG and FAI values across five age categories. SHBG levels were found to be higher with increasing age. Although TT and FAI were lower with increasing age, there was no significant difference between age groups. Similar to that observed for FT, the lowest level of TT was noted in the oldest age group.

Figure 5 demonstrates mean TT levels, SHBG and FAI across BMI categories. The lowest TT, SHBG and FAI were noted in the highest BMI category (>40 kg/m^2). Mean TT in the Study group with BMI >40 kg/m^2 was significantly lower when compared to patients in all other BMI categories [vs. <25 kg/m^2 ($p<0.0001$); vs. 25-29.9 kg/m^2 ($p<0.0001$); vs. 30-39.9 kg/m^2 ($p<0.05$)]. With respect to FT levels, Figure 6 shows mean serum FT values across BMI categories. Mean FT was lowest in the

> 40 kg/m^2 category and significantly lower when compared to all other BMI categories [vs. <25 kg/m^2 ($p<0.001$); vs. 25-29.9 kg/m^2 ($p<0.0001$); vs. 30-39.9 kg/m^2 ($p<0.05$)]. Study group participants with a BMI between 30 and 39.99 kg/m^2 also had significantly lower FT compared to patients with a BMI between 25-29.99 kg/m^2 ($p<0.01$).

5.2 Comparison between Study group and Control group

When compared with the Control group, mean serum TT (14.5 ± 5.8 vs. 18.8 ± 7.2 nmol/l; $p<0.001$), FAI (40.4 ± 17.0 vs. 49.6 ± 19.5; $p=0.002$) and FT (265.9 ± 90.4 vs. 351.7 ± 127.3 pmol/l; $p<0.001$) was significantly lower in the Study group (T2DM) while mean serum LH level was significantly higher (5.8 ± 3.1 vs 4.5 ± 2.3 IU/L; $p=0.006$) (Table 4). Of note is that serum LH in both groups was in the normal range.

Table 5 shows the prevalence of LSTT and LSFT in the Study group compared with the Control group. There was a significantly higher prevalence of LSTT and LSFT in the Study group compared to the Control group ($p=0.0087$ and $p=0.0088$, respectively).

Further analysis was performed to determine whether the differences in FT levels between study subjects and controls remained significant after adjusting for other variables that differed between these groups. Multiple variable logistic regression analysis was performed and factors entered into the model included age, BMI, LH levels and free androgen index (Table 6). After controlling for these variables, FT remained significantly lower in Study group vs. Control subjects ($p=0.001$) (Table 6).

Table 5: Prevalence of low serum total testosterone (LSTT) and low serum free testosterone (LSFT) in the study group (n=148) compared to the control group (n=50)

	%(n)		
	Study Group (n=148)	Control Group (n=50)	p*
LSTT	35.8 (53)	16.0 (8)	0.0087
LSFT	16.2 (24)	2.0 (1)	0.0088

LSFT: low serum free-testosterone; LSTT: Low serum total-testosterone; * Pearson chi square p value

Table 6: Multiple variable logistic regression analysis for independent association of free testosterone in the study group compared to the control group

		B	S.E.	Wald	df	Sig.	OR	95% CI for OR	
								Lower	Upper
Step 3[a]	Age	.136	.028	23.090	1	.000	1.146	1.084	1.211
	FAI	.064	.022	8.902	1	.003	1.067	1.022	1.113
	FT	-.011	.003	10.193	1	.001	.989	.983	.996
	LH	.220	.120	3.393	1	.065	1.246	.986	1.575
	Constant	-6.081	1.859	10.701	1	.001	.002		

[a] Variable(s) entered on step 1: Age, BMI, TT, FAI, FT, LH

BMI: body mass index, FAI: free androgen index, FT: free testosterone, LH: luteininsing hormone, TT: total testosterone,

Figure 3 : Serum free-testosterone in the study group, by age

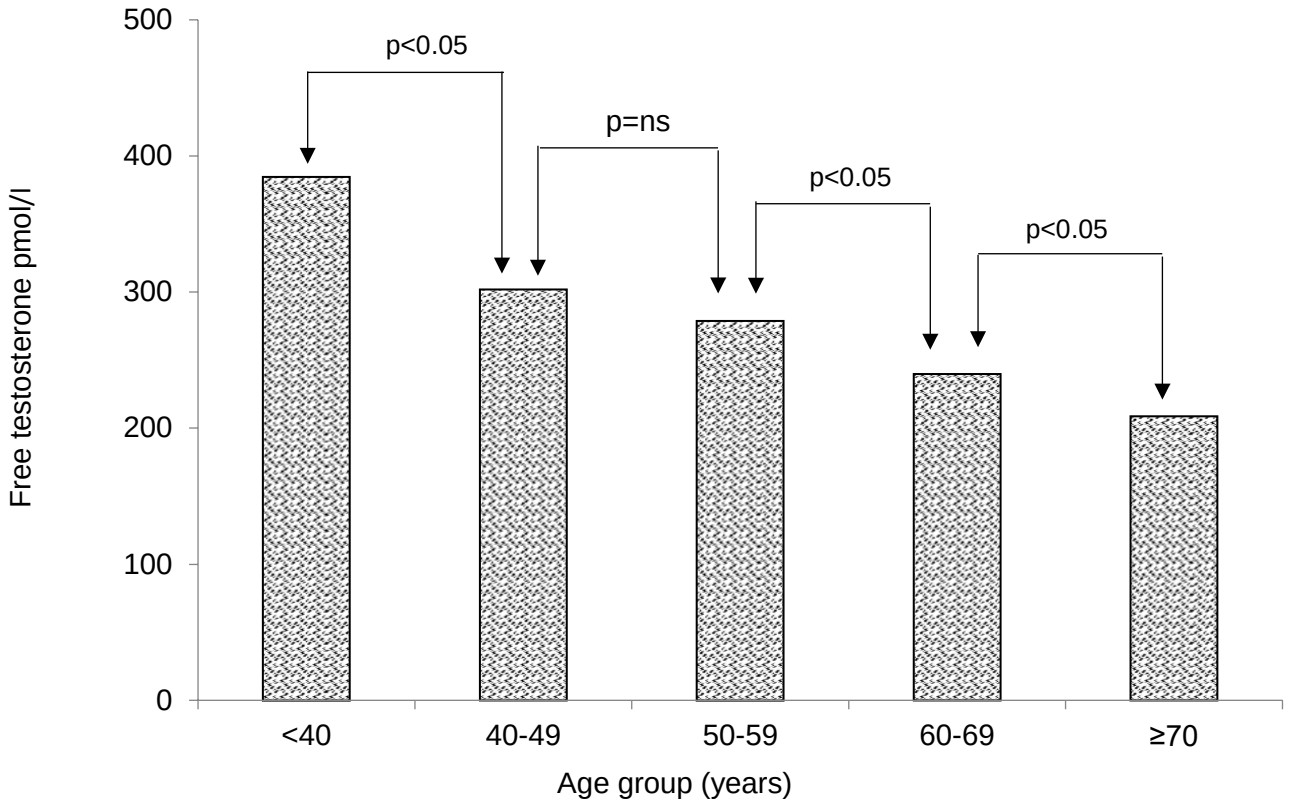


Figure 4a: Serum total testosterone (TT) in the study group, by age

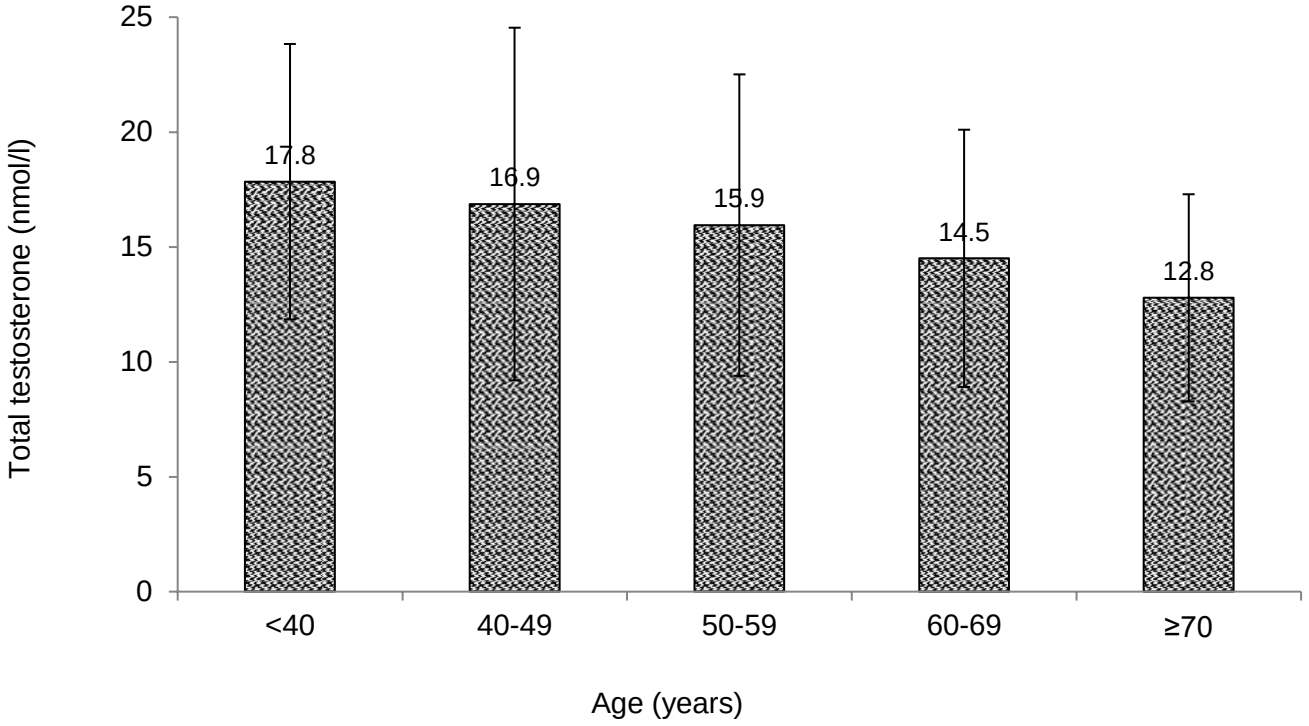


Figure 4b: Serum sex-hormone binding globulin (SHBG) levels in the study group, by age

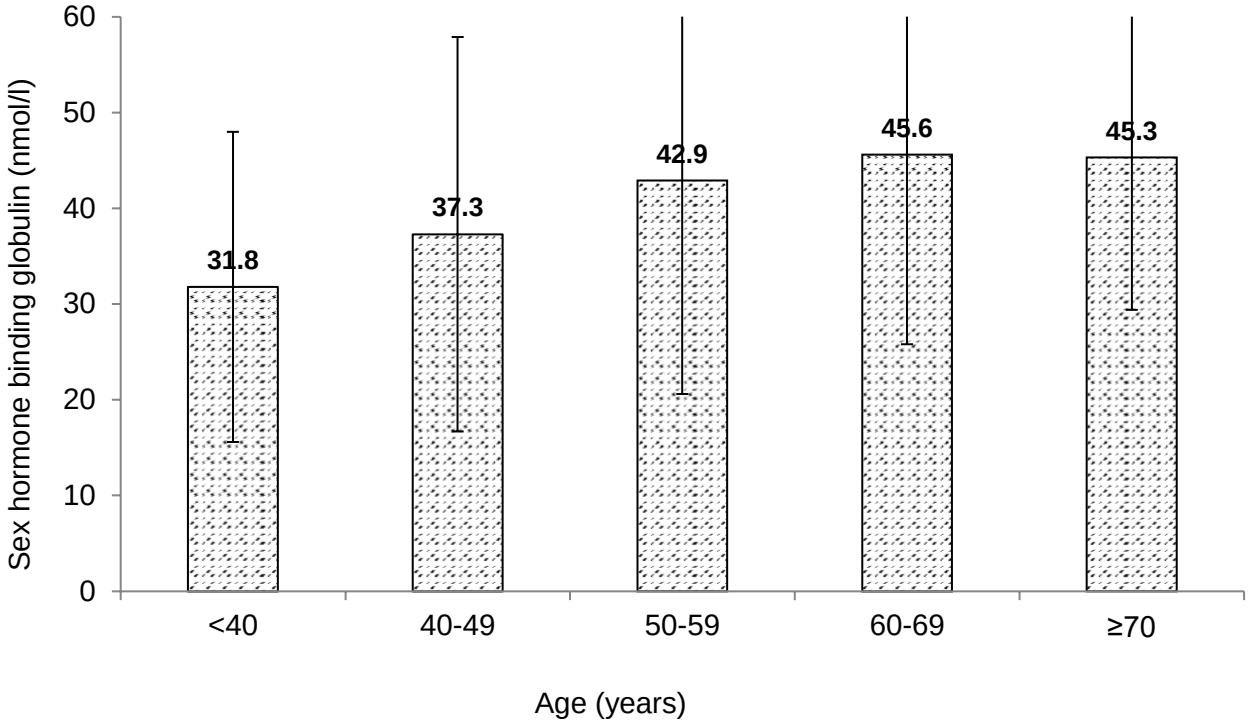


Figure 4c: Free androgen index (FAI) in the study group, by age

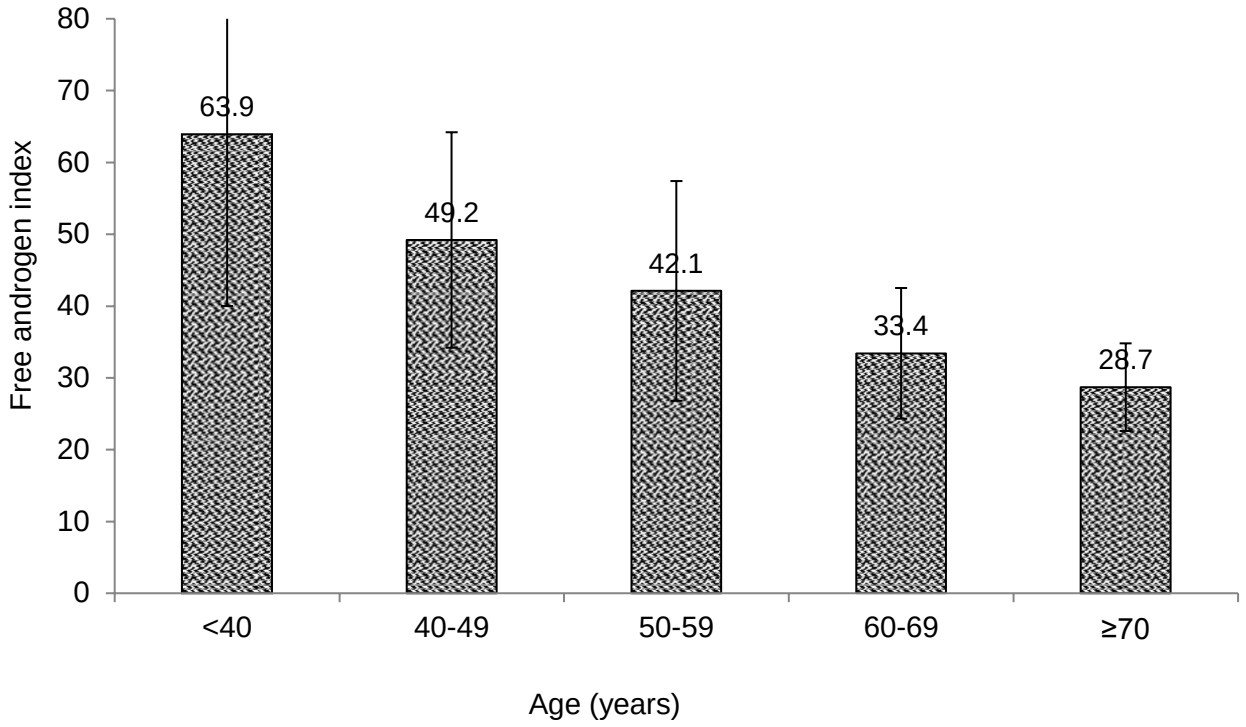


Figure 5a: Mean serum total-testosterone in the study group, by BMI category

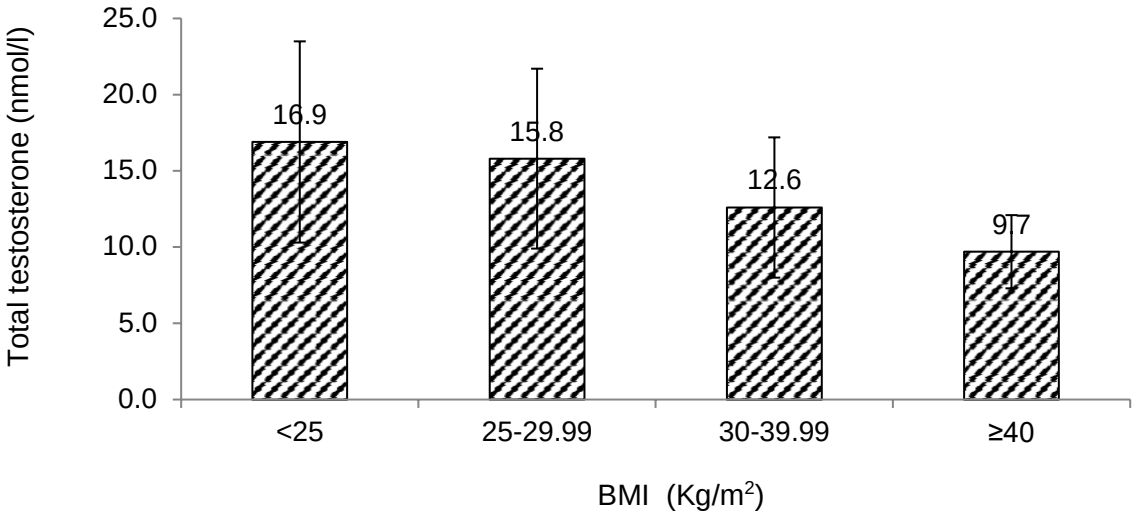


Figure 5B: Mean sex-hormone binding globulin (SHBG) in the study group, by BMI category

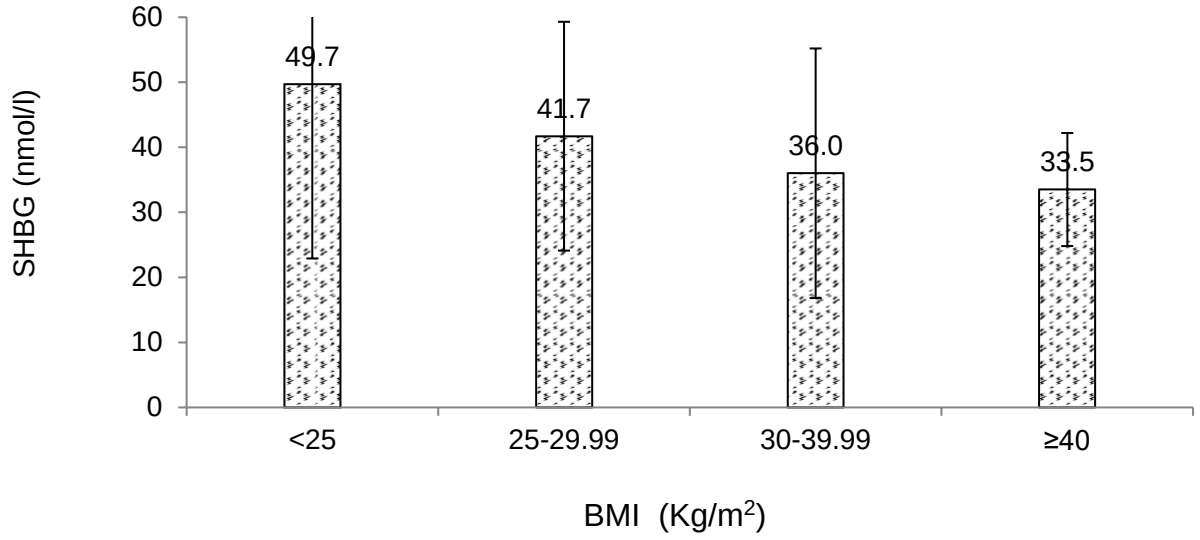


Figure 5C: Free androgen index in the study group, by BMI category

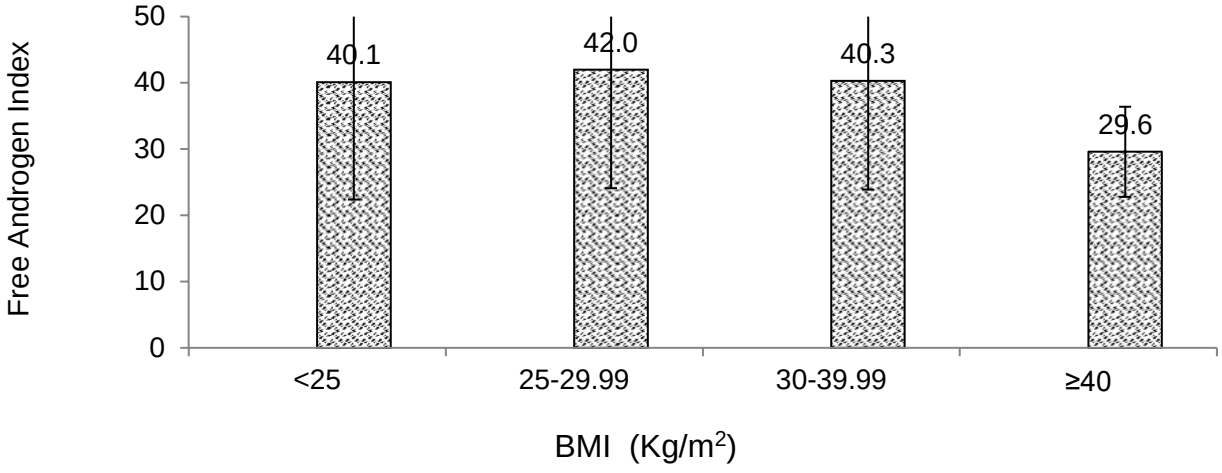
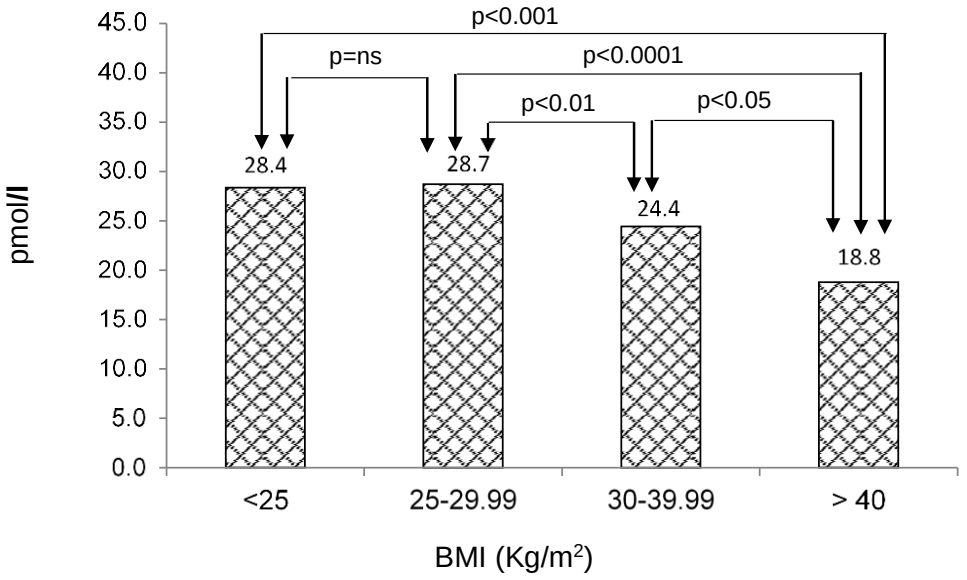


Figure 6: Mean serum free-testosterone across body mass index (BMI) categories



CHAPTER 6: PREVALENCE OF ANDROGEN DEFICIENCY SYMPTOMS AND AGEING MALE'S SYMPTOM (AMS) SCORE IN THE STUDY GROUP

The majority of the Study group reported a history of erectile dysfunction (58.8%, n=87) (Table 7). Loss of libido was also a common symptom, reported in 56.1% (n=83) of patients. Decrease in beard growth (2.7%) and infertility (2.0%) were infrequently reported.

Regarding the AMS questionnaire score (AMS) in the Study group, the median total score was 34 (26.5 - 42), with scores of 8.0 (5 - 11) for the psychological domain, 14 (11 - 19) for the somatic domain and 12 (9 - 15) for the sexual domain (Table 7). Twenty-five percent of patients had an AMS score that was < 27 and not consistent with symptoms of low serum testosterone. The overall prevalence of a high AMS score (≥ 27) was 74.5% (n=111). Of these, 36.2% (n=54) had a score of between 27 and 36, consistent with mild symptoms of low serum testosterone; 26.9% (n=40) scored between 37 and 49 i.e. moderate symptoms and 11.4% (n=17) scored above 49, consistent with severe symptoms.

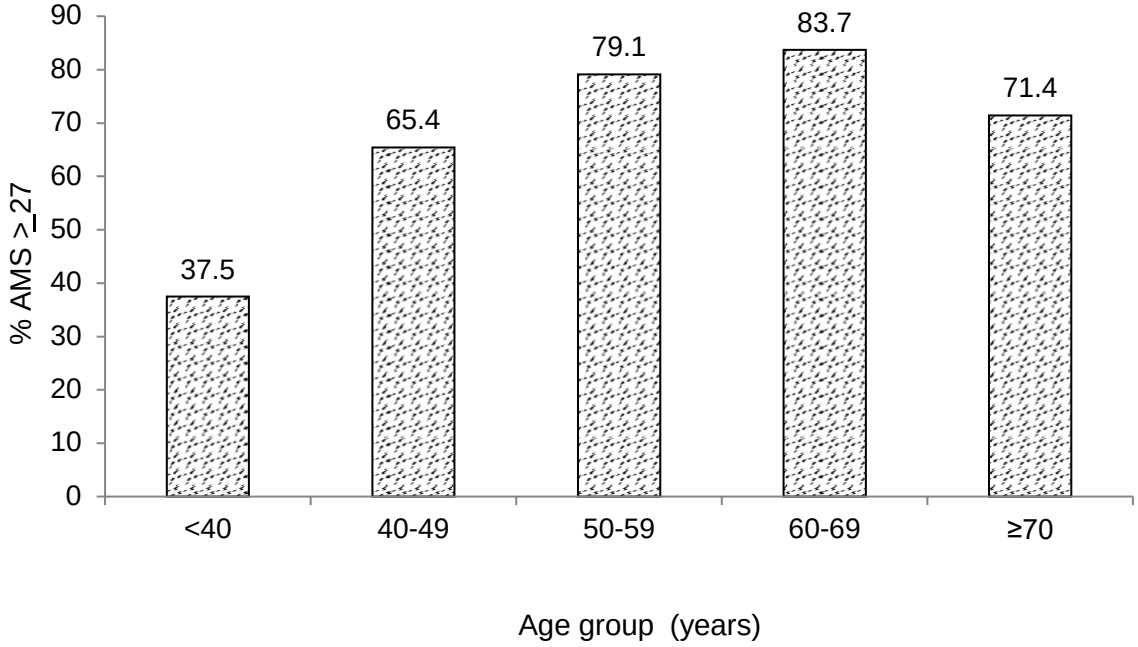
The prevalence of a high AMS score ≥ 27 varied across age groups. The highest prevalence was seen in the 60-69 year age category (83.6%). However, similarly high prevalence was also noted in the 40-49 year category (65.4%), 50-59 year age category (79.1%) and in 71.4% of patients in the >69 year age category. The lowest prevalence of 37.5% was seen in the <40 year age group (Figure 7).

Table 7: Prevalence of androgen deficiency symptoms in the study group (n:148)

	Study group (n=148)	
Symptoms on Enquiry	n	%
Any symptom	102	68.9
Erectile dysfunction	87	58.8
Low libido	83	56.1
Decrease in beard growth	4	2.7
Infertility	3	2.0
AMS questionnaire score*		
Psychological domain	5-11	8.0
Somatic domain	11-19	14.0
Sexual domain	9-15	12.0
Total score	26.5-42	34.0
High AMS score ≥ 27	111	75.0
AMS score 27-36	54	36.2
AMS score 37-49	40	26.9
AMS score >49	17	11.4

*Results expressed as Median (IQR) or n (%)
AMS: Aging males' symptom score; IQR: interquartile range

Figure 7:Prevalence of a positive Ageing Males' symptom (AMS) score ≥27 across age groups



CHAPTER 7: PREVALENCE OF LOW SERUM FREE TESTOSTERONE (LSFT), LOW SERUM TOTAL TESTOSTERONE (LSTT) AND HYPOGONADISM IN THE STUDY GROUP

Table 8 shows the prevalence of low serum testosterone and hypogonadism in the Study group.

7.1 Prevalence of low serum calculated free testosterone (LSFT)

The prevalence of low serum calculated free-testosterone (LSFT) was 16.2% (n=24) (Table 8) and varied across age groups and BMI (Figure 8 and 9). The prevalence was higher with age and the highest prevalence was noted in patients >60 years of age. The prevalence of LSFT was only 8.7% in the 40-49 year age category and in 0% of patients < 40 years of age (Figure 8). Figure 9 shows the varying prevalence of LSFT across BMI categories with the highest prevalence seen in patients with BMI >40 kg/m^2 (50%). However, LSFT was also present in the lowest BMI category (<25 kg/^{2}), with a prevalence of 14.8%.

7.2 Prevalence of low serum total testosterone (LSTT)

Using TT as the criterion for low testosterone, the prevalence was variable, depending on the serum TT cut-point. The prevalence of LSTT was 35.8% (n=53) with a cut-point of <12nmol/l; 10.1% (n=15) with TT values <8nmol/l and 25.7% (n=38) for borderline TT (8-12 nmol/l). Using the Endocrine Society clinical guideline (FT and TT), the prevalence of low testosterone was 18.9% (Table 8).

7.3 Prevalence of Hypogonadism

The prevalence of hypogonadism in the Study group was variable, depending on whether FT or TT was used the criterion for defining low serum testosterone (Table 8). Using FT as the criterion, the prevalence of hypogonadism was 11.5% (n=17). When TT < 12 nmol/l was used as the criterion for defining low testosterone, the prevalence was 26.3% (n=39). The prevalence of *borderline* hypogonadism was 18.9% (n=28) and that of *overt* hypogonadism was 7.4% (n=11) (Table 8).

The prevalence of primary versus secondary hypogonadism as defined by serum LH levels, among patients with LSFT and LSTT in this population are discussed in Chapter 12.

Table 8: Prevalence of low serum testosterone and hypogonadism in the study group (n:148)

	Study Group n=148	
Definition	n	%
Low serum free-testosterone (FT) <180 pmol/l	24	16.2
Low serum total-testosterone (TT)		
<12 nmol/l	53	35.8
<8 nmol/l	15	10.1
Borderline 8-12 nmol/l	38	25.7
Endocrine Society Definition*	28	18.9
Hypogonadism		
FT <180 pmol/l + AMS score ≥ 27	17	11.5
TT <12 nmol/ + AMS score ≥ 27	39	26.3
TT 8-12 nmol/ + AMS score ≥ 27**	28	18.9
TT <8 nmol/l + AMS score ≥ 27†	11	7.4

*Endocrine Society Clinical Practice Guideline definition of low serum testosterone (Bhasin et al.; 2010): TT < 8 nmol/l and TT 8-12 nmol/l with FT<180 pmol/l;
**Possible hypogonadism; † Overt hypogonadism; AMS: Ageing Males' Symptom Score

Figure 8: Prevalence of low serum free testosterone (LSFT) in the study group by age (n:147)

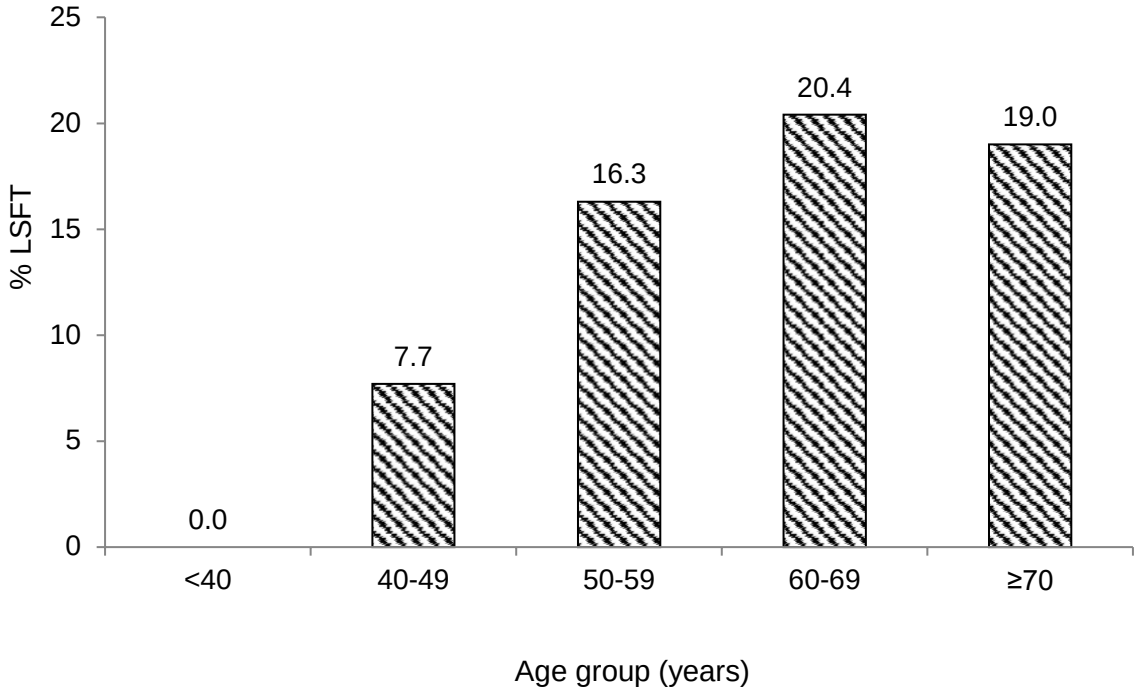
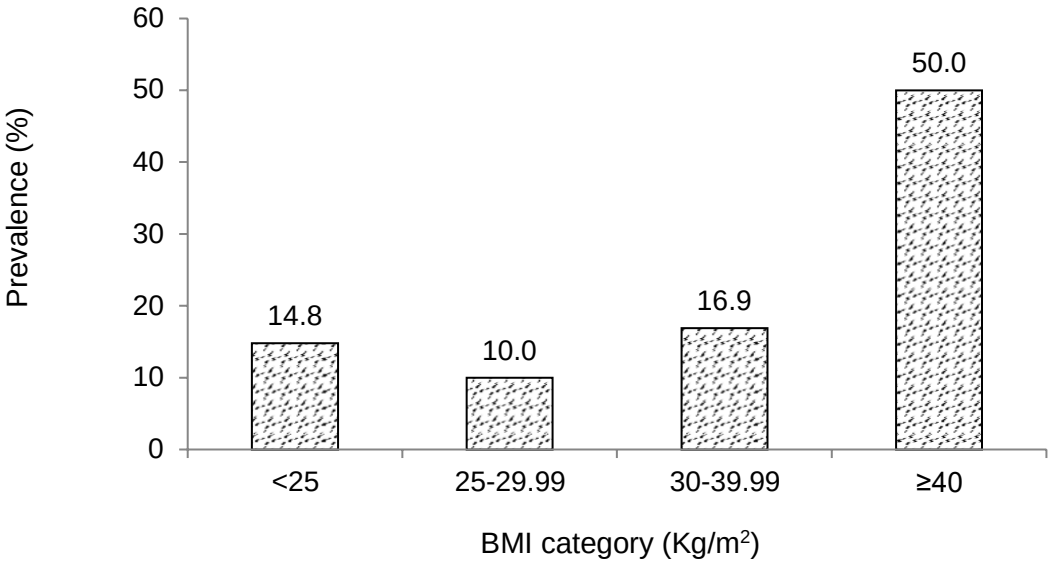


Figure 9 : Prevalence of low serum free testosterone (LSFT) in the study group, by BMI categories



CHAPTER 8: CLINICAL AND BIOCHEMICAL CHARACTERISTICS IN THE STUDY GROUP WITH NORMAL SERUM FREE TESTOSTERONE (NSFT) AND LOW SERUM FREE TESTOSTERONE (LSFT) AND RISK FACTORS FOR LSFT

There was no significant difference in the total or individual domain AMS questionnaire scores between patients with LSFT and those with NSFT. The prevalence of an elevated AMS score (≥ 27) was 70.8% (n=17) in patients with LSFT compared to 75.8% (n=94) in patients with NSFT (Table 9).

Tables 10 and 11 show the relationship between LSFT and variables including demographic, clinical and laboratory variables. Variables that were found to be significantly associated with LSFT in univariate analysis (Table 10) included age, decrease in beard growth, BMI, waist circumference, hip circumference and albumin level. In multivariate logistic regression analysis (Table 11), the significant independent risk factors associated with LSFT included age [OR 1.05 (95%CI 1.02-1.218), p=0.043)] and waist circumference [OR 1.033 (95%CI 0.999-1.068, p=0.059)]. For every increase in age by 1 year, the risk of LSFT was higher by 5%. For every 1cm increase in waist circumference, the risk of LSFT was higher by 33% (Table 11).

Receiver operating characteristic (ROC) analysis was performed using the model in Table 11 to predict the probability of LSFT; the area under the curve (AUC) was 0.658. The overall percentage of cases correctly predicted by the model was 84.4% (Figure 10).

Table 9: Ageing Males' Symptom (AMS) score in the study group: patients with normal serum free testosterone (NSFT) [n:24] vs. low serum free testosterone (LSFT) [n:124]

AMS score	LSFT (n:24)	NSFT (n:124)	p
Psychological domain	6.0 (5-9)	8.0 (5-11)	0.4
Somatic domain	11.5 (11-20)	14.0 (11-18)	0.3
Sexual domain	11.5 (7.5-15)	12.0 (9-15)	0.5
Total score	36.0 (23-43.5)	34.0 (27-42)	0.9
AMS score ≥ 27 [%(n)]	70.8 (17)	75.8 (94)	0.6

Results are median (IQR) or % (n)*; AMS score: Ageing Males' Symptom score; NSFT: normal serum free-testosterone; LSFT: low serum free-testosterone

Table 10: Clinical risk factors and hormonal characteristics associated with low serum calculated free testosterone (LSFT) in the study group (n:148)

	LSFT	NSFT	OR	95% CI	P *
Demographics					
Age	61.4 ± 10.4	56.8 ± 11.2	1.050	1.004-1.099	0.033 *
Duration DM	12.0 ± 10.9	11.3 ± 8.6	1.009	0.962-1.058	0.718
Medical History					
Erectile dysfunction	13 (54.2)	74 (59.2)	1.138	0.463-2.798	0.777
Loss of Libido	8 (33.3)	75 (60)	2.870	1.132-7.278	0.026 *
Decreased beard growth	2 (8.3)	2 (1.6)	0.172	0.023-1.290	0.087 *
Infertility	1 (4.2)	2 (1.6)	0.361	0.031-4.150	0.413
Retinopathy	8 (33.3)	27 (22)	1.796	0.695-4.644	0.227
Microalbuminuria	8 (33.3)	26 (21)	1.885	0.727-4.885	0.192
Proteinuria	7 (29)	20 (16)	2.141	0.786-5.831	0.136
IHD	3 (13)	23 (19)	0.627	0.172-2.283	0.497
PVD	3 (13)	8 (6)	2.071	0.508-8.450	0.310
CVD	2 (8)	4 (3)	2,727	0.471-15.808	0.263
Smoker (current)	7 (29.2)	24 (19.4)	1.541	0.557-4.26	0.404
Smoker (ex)	3 (12.5)	21 (16.9)	0.755	0.198-2.877	0.681
Clinical					
Height	1.7 ± 0.1	1.7 ± 0.1	0.009	0-19.9	0.227
Weight	90.4 ± 25.3	85 ± 15.9	1.016	0.993-1.040	0.174
BMI	31.9 ± 8.6	29.4 ± 5.3	1.063	0.994-1.136	0.073 *
Waist circumference	108.3 ± 17.7	102.3 ± 11.7	1.035	0.110-1.069	0.041 *
Hip circumference [#]	111.0 ± 14.5	105.4 ± 8.3	1.048	0.993-1.107	0.086 *
WHR [#]	1.0 ± 0.08	0.97 ± 0.07	63.216	0.031-12947	0.286
Systolic BP	134.4 ± 15.5	135,.9 ± 16.7	0.994	0.968-1.021	0.675
Diastolic BP	76.1 ± 11.5	77.5 ± 97	0.986	0.943-1.030	0.532
Laboratory tests					
HbA1c	8.6 ± 1.7	8.6 ± 2.0	1.005	0.797-1.265	0.968
Total cholesterol	4.3 ± 1.5	4.2 ± 1.3	1.020	0.733-1.421	0.904

Triglycerides	1.9 ± 0.9	2.1 ± 1.5	0.893	0.634-1.258	0.517
HDL-cholesterol	1.1 ± 0.2	1.1 ± 0.3	1.069	0.182-6.278	0.941
LDL-cholesterol	2.2 ± 1.5	2.2 ± 0.9	1.0	0.645-1.550	0.999
Haemoglobin	13.2 ± 1.5	13.4 ± 1.7	0.923	0.691-1.231	0.585
Haematocrit	0.4 ± 0.04	0.4 ± 0.04	0.007	0-288	0.359
Albumin	42.5 ± 3.5	44.5 ± 4.5	0.902	0.810-1.004	0.058 *
ALP	109.9 ± 112	90.5 ± 28.8	1.005	0.997-1.013	0.213
GGT	94.7 ± 242.7	50.3 ± 46.8	1.003	0.999-1.008	0.203
ALT	28.7 ± 23.9	31.8 ± 21.9	0.992	0.966-1.019	0.571
AST	27.6 ± 13.8	25.2 ± 97	1.019	0.978-1.061	0.364
Metabolic syndrome	22 (92)	105 (85)	1.990	0.432-9.170	0.377

Results are mean ± SD or n(%); *Significance at 0.1 level; # missing replaced with series mean.
IHD: ischaemic heart disease, PVD: peripheral vascular disease, CVD: cerebrovascular disease, BMI: body mass index, BP: blood pressure, ALP: alkaline phosphatase, GGT: gammaglutamyl transferase, ALT: alanine transaminase, AST: aspartate transaminase, HbA_{1c}: haemoglobin A1c, LDL: low density lipoprotein, HDL: high density lipoprotein, WHR: waist to hip ratio

Table 11: Multivariate logistic regression analysis for the clinical risk factors and hormonal characteristics associated with low serum calculated free testosterone levels in the study group

		B	S.E.	Wald	df	Sig.	OR	95% CI for OR	
								Lower	Upper
Step 4[a]	Age	.049	.024	4.108	1	.043	1.050	1.002	1.100
	WC	.033	.017	3.565	1	.059	1.033	.999	1.068
	Constant	-8.013	2.402	11.126	1	.001	.000		

[a.] Variable(s) entered on step 1: Albumin, Hip circumference (HC), Age, Body mass index (BMI), Waist circumference (WC)

Figure 10: Reciever operating characteristics (ROC) curve – model using age and waist circumference for predicting low serum free testosterone (LSFT) levels

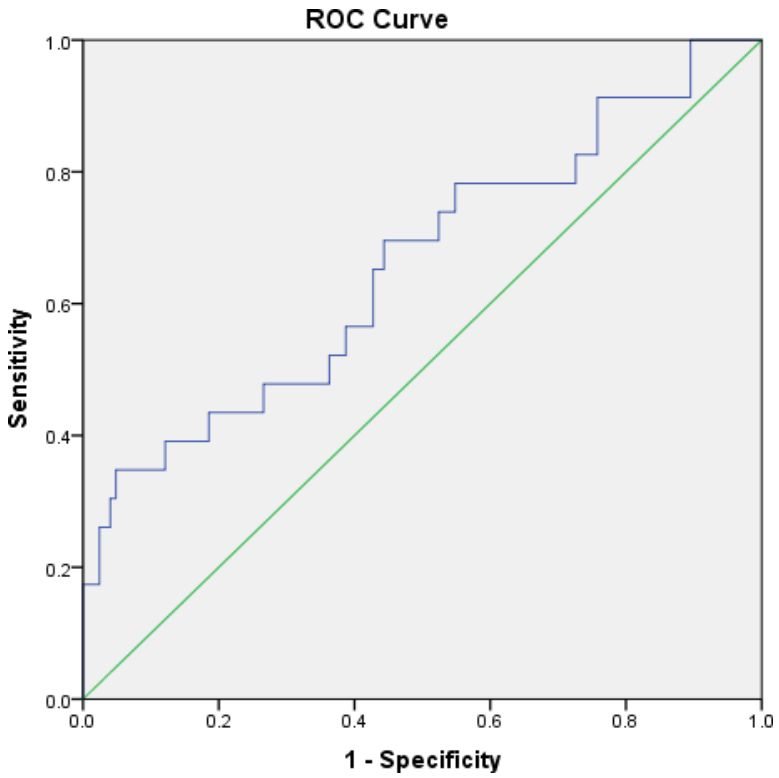


Figure 10. Reciever operating characteristic (ROC) analysis was performed using age and waist circumference in a model to predict the probability of low serum free testosterone (LSFT). The area under the curve (AUC) was 0.658. The overall percentage of cases correctly predicted by the model was 84.4%.

CHAPTER 9: CLINICAL AND BIOCHEMICAL CHARACTERISTICS IN THE STUDY GROUP WITH NORMAL SERUM TOTAL TESTOSTERONE (NSTT) AND LOW SERUM TOTAL TESTOSTERONE (LSTT) AND RISK FACTORS FOR LSTT

Table 12 shows the comparison between patients with LSTT and NSTT with respect to demographic data, clinical variables, laboratory results and risk factor analysis. When compared with NSTT, patients with LSTT had a higher prevalence of peripheral vascular disease (PVD) and measures of adiposity. Univariate logistic regression analysis found a significant association between LSTT and the presence of microalbuminuria, presence of proteinuria, weight, BMI, waist circumference, hip circumference and the presence of metabolic syndrome. In multivariate logistic regression analysis, the only significant risk factor associated with LSTT was BMI [OR 1.138 (95%CI 1.063-1.218), p<0.0001)] (Table 13). For every one unit increase in BMI, the risk of LSTT was higher by 1.138 times (p<0.001).

ROC analysis was performed using this model to predict the probability of LSTT and gave an area under the curve (AUC) of 0.677. The overall percentage of cases correctly predicted by the model was 70.3% (Figure 11).

Table 12: Clinical risk factors and hormonal characteristics associated with low serum total testosterone levels (LSTT) in the study group (n:148)

	LSTT	NSTT	OR	95% CI	P
Demographics					
Age	58.0 ± 11.7	57.5 ± 11.0	1.008	0.977-1.039	0.615
Duration DM	11.5 ± 10.2	11.4 ± 8.3	1.002	0.965-1.040	0.906
Medical History					
Erectile dysfunction	22.0 (41.5)	59 (62.1)	1.405	0.708-2.786	0.331
Loss of Libido	20 (37.7)	59 (62.1)	1.912	0.964-3.793	0.064 *
Decreased beard growth	2 (3.8)	2 (2.1)	0.538	0.074-3.933	0.541
Infertility	1 (1.9)	2 (2.1)	1.097	0.097-12.391	0.940
Retinopathy	9 (17)	23 (24.2)	0.916	0.413-2.032	0.830
Microalbuminuria	11 (20.8)	17 (17.9)	2.167	0.944-4.725	0.052 *
Proteinuria	9 (71)	13 (13.7)	2.264	0.972-5.275	0.058 *
IHD	8 (15.1)	15 (15.8)	1.397	0.589-3.311	0.448
PVD	7 (13.2)	3 (3.2)	5.452	1.380-21.540	0.016
CVD	2 (3.8)	3 (3.2)	1.840	0.358-9456	0.465
Smoker	10 (18.9)	21 (22.1)	0.794	0.333-1.890	0.602
Smoker (ex)	8 (15.1)	15 (15.8)	1.000	0.394-2.504	1.000
Clinical					
Height	1.7 ± 0.1	1.7 ± 0.1	1.029	0.004-297.4	0.992
Weight	93.7 ± 21.4	81.5 ± 13.7	1.043	1.020-1.066	<0.001 *
BMI	32.5 ± 7.4	28.3 ± 4.5	1.138	1.063-1.218	<0.001 *
Waist circumference	108.5 ± 14.9	100.4 ± 10.7	1.053	1.022-1.084	0.001 *
Hip circumference #	110.7 ± 10.5	103.6 ± 7.7	1.099	0.034-1.168	0.002 *
WHR #	1.0 ± 0.1	1.0 ± 0.07	17.307	0.039-7620	0.359
Systolic BP	135.2 ± 15.4	135.9 ± 17.1	0.998	0.977-1.018	0.818
Diastolic BP	77.1 ± 9.3	77.4 ± 10.4	0.998	0.964-1.032	0.885
Laboratory tests					
HbA1c	8.7 ± 1.8	8.5 ± 2.0	0.031	0.863-1.231	0.738
Total cholesterol	4.4 ± 1.3	4.2 ± 1.3	1.103	0.854-1.424	0.452

Total triglycerides	2.3 ± 1.4	2.0 ± 1.5	1.157	0.916-1.462	0.222
HDL-cholesterol	1.0 ± 0.2	1.1 ± 0.3	0.506	0.123-2.093	0.347
LDL-cholesterol	2.3 ± 1.2	2.1 ± 0.9	1.191	0.842-1.687	0.323
Haemoglobin	13.2 ± 1.6	13.5 ± 1.7	0,890	0.712-1.114	0.309
Haematocrit	0.4 ± 0.05	0.4 ± 0.04	0.001	0-4.8	0.112
Albumin	43.6 ± 4.2	44.5 ± 4.4	0.954	0.874-1.041	0.292
ALP	97.4 ± 79.3	91.8 ± 30	1.002	0.995-1.009	0.599
GGT	68.1 ± 166.8	52.8 ± 50.1	1.001	0.996-1.005	0.477
ALT	29.5 ± 20.0	32.3 ± 23.5	0.994	0.975-1.013	0.519
AST	25.8 ± 11.2	25.5 ± 10.1	1.003	0.967-1.039	0.891
Metabolic syndrome	49 (92.5)	78 (82.1)	2.670	0.848-8.401	0.093 *

Results are mean ± SD or n(%); * Significance at 0.1 level; # missing replaced with series mean.
Abbreviations: IHD: ischaemic heart disease, PVD: peripheral vascular disease, CVD: cerebrovascular disease, BMI: body mass index, BP: blood pressure, ALP: alkaline phosphatase, GGT: gammaglutamyl transferase, ALT: alanine transaminase, AST: aspartate transaminase, HbA_{1c}: haemoglobin A1c, LSTT: low serum total testosterone; NSTT: normal serum total testosterone, WHR: waist to hip ratio

Table 13: Multivariate logistic regression analysis showing the clinical risk factors and hormonal characteristics associated with low serum total testosterone (LSTT) levels in the study group

		B	S.E.	Wald	df	Sig.	OR	95% CI for OR	
								Lower	Upper
Step 4[a]	BMI	.129	.035	13.800	1	.000	1.138	1.063	1.218
	Constant	-4.479	1.068	17.599	1	.000	.011		

a. Variable(s) entered on step 1: metabolic syndrome, hip circumference, waist circumference, microalbuminuria, proteinuria, weight, BMI

Figure 11: Reciever operating characteristics (ROC) curve – model using body mass index for predicting low serum total testosterone (LSTT) levels

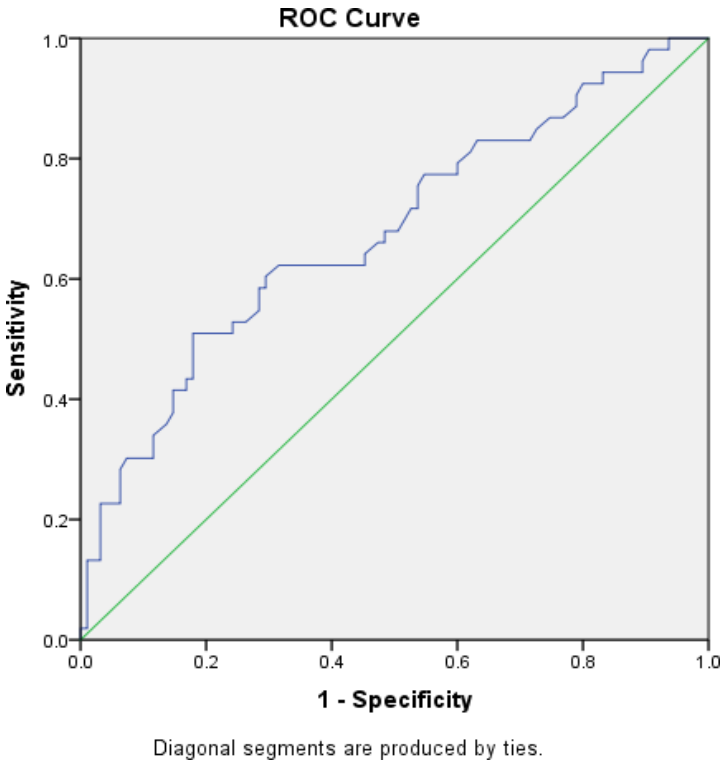


Figure 11. Reciever operating characteristic (ROC) analysis was performed using body mass index to predict the probability of low serum total testosterone (LSTT). The area under the curve (AUC) was 0.677. The overall percentage of cases correctly predicted by the model was 70.3%.

CHAPTER 10: VALUE OF THE AGEING MALE'S SYMPTOM (AMS) QUESTIONNAIRE IN PREDICTING LOW SERUM FREE TESTOSTERONE (LSFT) AND LOW SERUM TOTAL TESTOSTERONE (LSTT) IN THE STUDY GROUP

The utility of the AMS questionnaire in predicting LSFT and LSTT was assessed using ROC analysis (Figure 12 and 13). None of the individual domains nor the total AMS score were a good predictor of either LSFT or LSTT. In both cases, the AUC was not significantly better than 0.5 in any of the scores or total score. Sensitivities were not calculated due to the poor predictive probability of the questionnaire. There was a low correlation between TT and the AMS scores and this was also the case for FT (Table 14).

Table 14: Pearson correlation between Ageing Males' Symptoms (AMS) score and serum total testosterone (TT) and free testosterone (FT) in the Study Group

		Psychological	Somatic	Sexual	Total Score
Total testosterone (TT)	Pearson Correlation	.097	-.007	.154	.087
	Sig. (2-tailed)	.243	.930	.062	.295
	N	148	148	148	148
Free Testosterone (FT)	Pearson Correlation	.109	-.104	.018	-.003
	Sig. (2-tailed)	.185	.209	.830	.974
	N	148	148	148	148

Figure 12: ROC curve results for predicting low serum free testosterone (LSFT) using the Ageing male's symptom (AMS) questionnaire

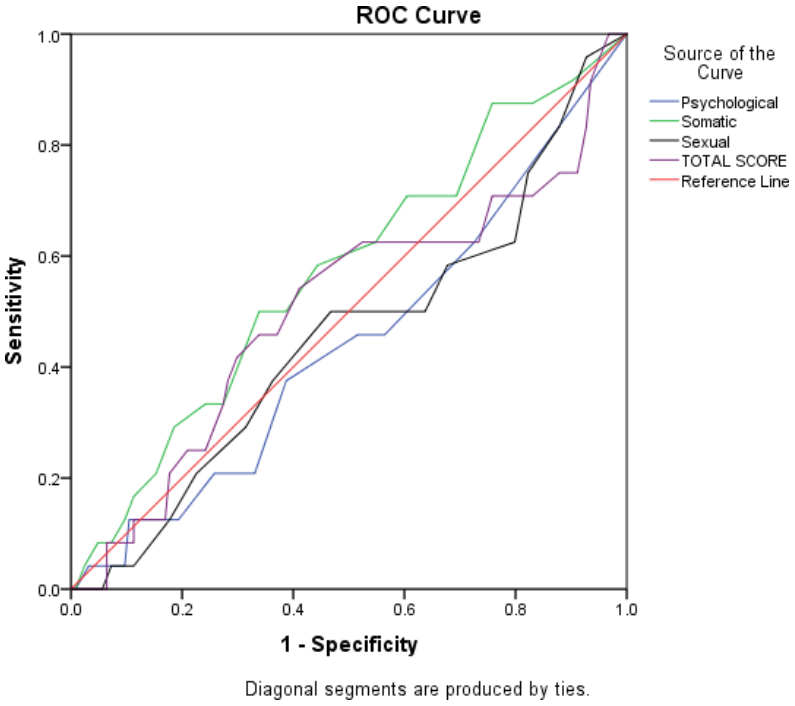


Figure 12. Reciever operating characteristic (ROC) analysis was performed using the Ageing male's symptom (AMS) questionnaire to predict the probability of low serum free testosterone (LSFT). The area under the curve (AUC) for individual components of the score and the total score was below 0.5.

Figure 13: ROC curve results for predicting low serum total testosterone (LSTT) using the Ageing male's symptom (AMS) questionnaire

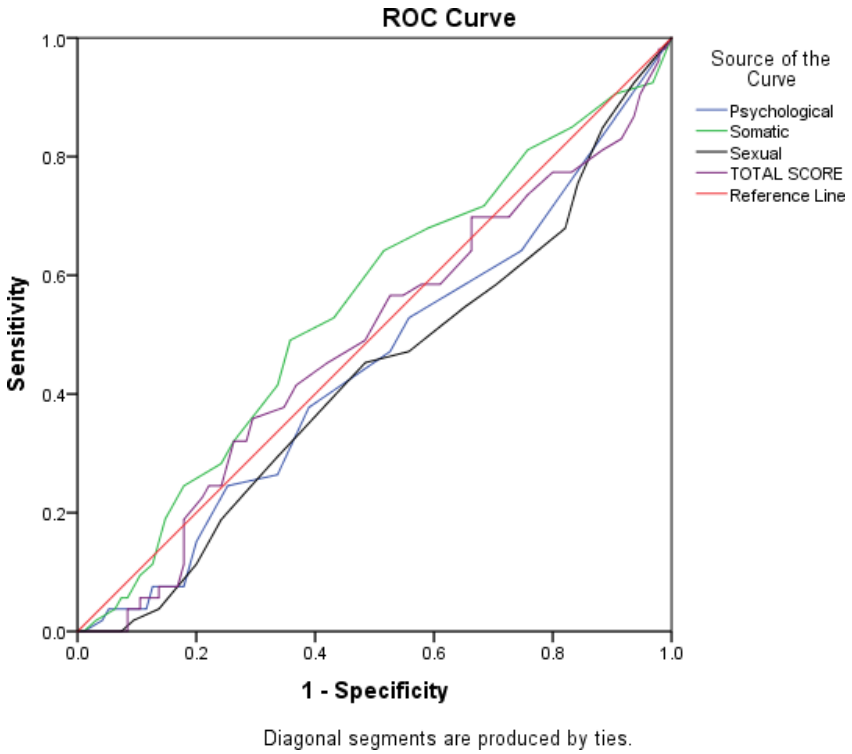


Figure 13. Reciever operating characteristic (ROC) analysis was performed using the Ageing male's symptom (AMS) questionnaire to predict the probability of low serum total testosterone (LSTT). The area under the curve (AUC) for individual components of the score and the total score was below 0.5.

CHAPTER 11: CORRELATION ANALYSIS IN THE STUDY GROUP

The correlation between the hormonal variables (TT, FT, FAI, SHBG) and clinical characteristics and metabolic characteristics in the study group are outlined in Tables 15 and 16 and Figure 14.

There was a moderate inverse correlation between BMI, waist circumference and number of features of the metabolic syndrome with TT ($r = -0.34$, $p<0.001$; $r=-0.35$, $p<0.001$; $r=-0.3$, $p<0.001$).

For FAI, weak correlations were observed with duration of DM, total daily insulin dose, WHR and waist circumference. A moderate inverse correlation was also noted between FAI and age ($r= -0.62$, $p<0.001$).

With respect to FT, there was a weak negative correlation with BMI, WHR and number of features of the metabolic syndrome ($r= -0.27$, $p<0.001$; $r= -0.22$, $p<0.05$; $r= -0.19$, $p<0.05$) but a moderate inverse correlation with age and waist circumference ($r= -0.45$, $p<0.001$; $r= -0.33$, $p<0.001$). The scatter plot (Figure 14) shows the negative correlation of FT with BMI and weight ($r= -0.27$, $p<0.001$; $r= -0.21$, $p<0.01$).

A moderate positive correlation was observed between SHBG and age ($r= 0.3$; $p<0.001$).

Table 16 shows the correlation between the metabolic and hormonal profile among the study subjects. There was a weak inverse correlation between serum triglycerides and TT (r = -0.18, p=0.03) but this relationship was not observed with FT. A weak inverse correlation was also noted between SHBG and serum triglycerides (r= -0.25, p=0.003). For both FT and TT, no significant correlation was observed with HbA_{1c}.

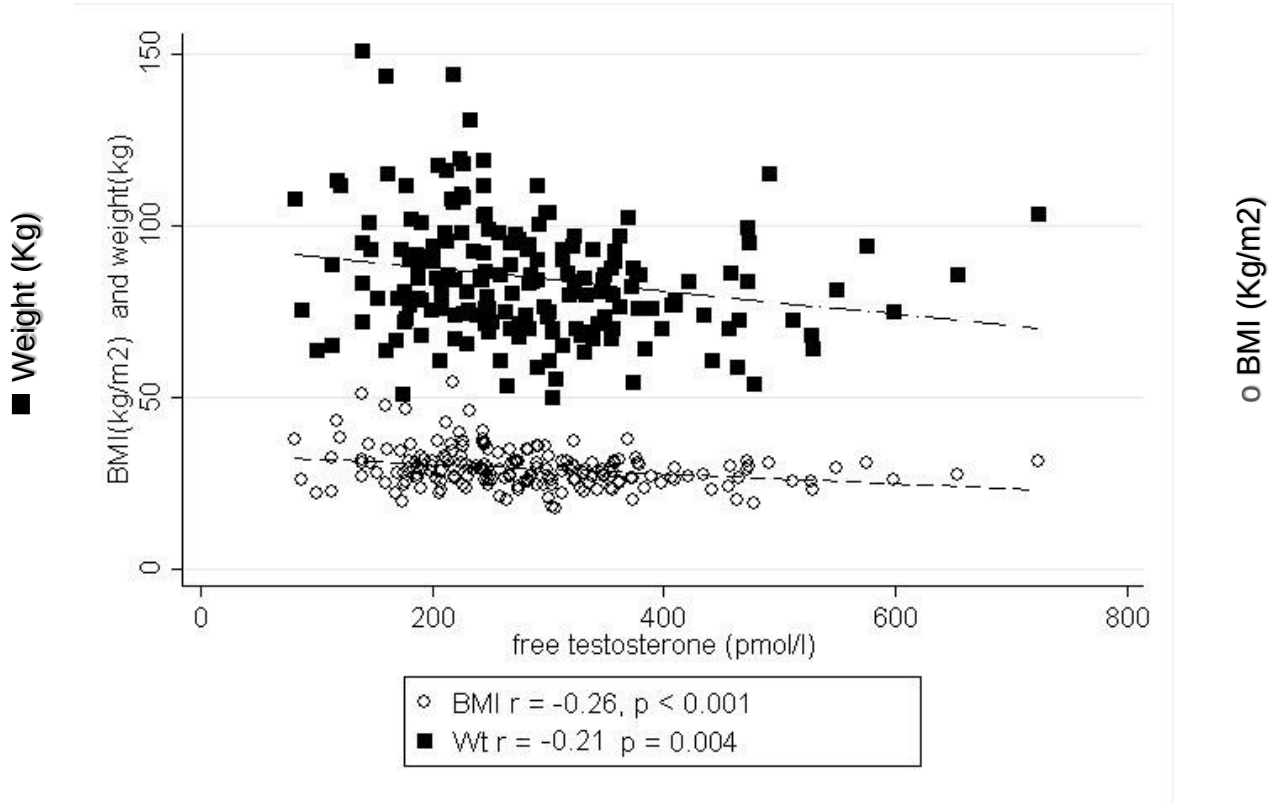
Table 15: Correlation between clinical characteristics and hormonal profile in the study group

	Total Testosterone (TT)		Free Testosterone (FT)		Sex-hormone binding globulin (SHBG)	
	r	p	r	p	r	P
Age	-0,07	0,4	-0,45	<0.001	0,33	<0.001
Duration of Diabetes	-0,06	0,46	-0,16	0,06	0,04	0,6
Insulin daily dose	-0,07	0,4	-0,15	0,08	0,03	0,7
Body mass index	-0,34	< 0.001	-0,27	<0.001	-0,20	0,01
Waist to hip ratio	-0,20	0,1	-0,22	0,04	-0,06	0,6
Waist circumference	-0,35	<0.001	-0,33	<0.001	-0,15	0,1
Number of features of Metabolic syndrome	-0,30	<0.001	-0,19	0,02	-0,24	0,003

Table 16: Correlation between the metabolic and hormonal profile in the study group

	Total Testosterone (TT)		Free androgen Index		Free Testosterone (FT)		SHBG	
	r	p	r	P	r	p	r	p
HbA1c	-0,10	0,2	-0,001	0,9	-0,02	0,78	-0,11	0,2
Total cholesterol	0,07	0,4	0,07	0,4	0,08	0,37	0,01	0,9
LDL cholesterol	0,01	0,9	0,05	0,6	0,02	0,80	-0,01	0,9
HDL cholesterol	0,13	0,1	0,03	0,7	0,10	0,28	0,09	0,3
Total Triglycerides	-0,18	0,03	0,10	0,2	-0,02	0,86	-0,25	0,003

Figure 14: Relationship between free-testosterone (FT) and body mass index (BMI) and weight in the study group



CHAPTER 12: SERUM LUTEINIZING HORMONE (LH) LEVELS IN THE STUDY GROUP AND CONTROL GROUP AND BY TESTOSTERONE CATEGORIES

Table 17 shows the mean LH level in the Study group and Control subjects by serum testosterone categories. Overall, when compared to control subjects, patients with T2DM had a significantly lower mean serum LH level (4.5 ± 2.3 IU/L vs. 5.8 ± 3.1; p=0.006). However, the mean LH levels were within the normal reference ranges both in the Study and Control groups.

When the Study group was divided into normal and low testosterone categories, there was no significant difference in mean LH levels between patients with LSFT vs. NSFT or LSTT vs. NSTT (Table 17).

Among patients with LSTT, in subjects with TT < 8nmol/l, the prevalence of patients with high, normal and low LH levels was 26.7% (n=45), 60% (n=9) and 13.3% (n=2), respectively (Table 18). In the group with borderline TT (8-12 nmol/l), the proportion of patients with high, normal and low LH levels were 15.8% (n=6), 78.9% (n=30) and 5.3% (n=2), respectively.

Table 18 shows the distribution of LH in the Study group with low testosterone levels. In patients with a LSFT (n=24), 20.8% (n=5) had elevated LH values, 70.8% (n=17) had normal levels and 8.3% (n=2) had low LH levels.

The prevalence of a low or normal LH level (compatible with secondary hypogonadism) among patients with LSFT was 79.2% and the remainder (20.8%) had elevated LH compatible with primary hypogonadism. The corresponding prevalence of a low or normal LH among patients with LSTT (< 12 nmol/l) was 81.1% and the remainder (18.9%) had elevated LH levels compatible with primary hypogonadism (Table 18).

Table 17: Serum luteinising hormone (LH) levels in the study group and control group and by serum testosterone categories

		Luteinising hormone (IU/L)	P
	Study group vs. Control group		
All			0.006
	Study group (n=148)	4.5 ± 2.3	
	Control group (n=50)	5.8 ± 3.1	
	Study group		
by Total testosterone			0.7
	LSFT group (n=124)	6.1 ± 4.2	
	NSFT group (n=24)	5.8 ± 3.9	
by Free testosterone			0.06
	LSTT group (n=95)	5.7 ± 3.7	
	NSTT group (n=53)	5.9 ± 2.8	

Results expressed as Mean ± SD. TT: total testosterone; FT: calculated free-testosterone; LSFT: low serum free testosterone; NSFT: normal serum free testosterone; LSTT: low serum total testosterone; NSTT: normal serum total testosterone, LH: luteinising hormone

Table 18: Serum luteinising hormone (LH) levels in the study group with low serum free-testosterone (LSFT) and low serum total testosterone (LSTT) levels

Testosterone category	Luteinising hormone (LH) n (%)		
	Low	Normal	High
Total testosterone (TT)			
TT < 12 nmol/l (n:53)	4 (7.5)	39 (73.6)	10 (18.9)
TT < 8 nmol/l (n:15)	2 (13.3)	9 (60)	4 (26.7)
TT 8-12 nmol/l (n:38)	2 (5.3)	30 (78.9)	6 (15.8)
Free testosterone (FT)			
FT <180 pmol/l (n:24)	2 (8.3)	17 (70.8)	5 (20.8)

LH (IU/L) range: low < 1.8 IU/L; normal 1.8-8.2 IU/L; high > 8.2IU/L

CHAPTER 13: DISCUSSION

In this study, in men with T2DM, there was a high prevalence of LSFT <180pmol/l (16.2%) and LSTT <12nmol/l (35.8%). Mean serum TT and mean serum FT were significantly lower in patients with T2DM compared to control subjects. The overall prevalence of androgen deficiency symptoms was 74.5% using the AMS questionnaire and 69% on direct patient enquiry, but this difference was not significant. Low serum testosterone was not associated with symptoms of androgen deficiency and there was a low correlation between AMS scores and both FT and TT. LH levels were either normal or low in the majority of patients with LSFT (79.2%) or LSTT (73.3%) compatible with hypogonadotropic hypogonadism, with the remainder having elevated levels more in keeping with primary hypogonadism.

In the Study group, in multivariate analysis, when compared to patients with normal serum total testosterone (NSTT), in patients with LSTT there was a significant independent negative association only with BMI; an independent negative association with age and waist circumference was found in patients with LSFT (compared to those with NSFT).

13.1. Prevalence of low serum testosterone

13.1.1 Global prevalence

In this study there was a high prevalence of LSFT (16.2%) and LSTT (35.8%) in men with T2DM. The findings of the current study are in accordance with reports from cross-sectional studies in western countries such as the United States that showed a high prevalence (17-33%) of low serum testosterone in men with diabetes (6,23). A high prevalence of low serum testosterone in men with T2DM has also been

reported from India (26.3%), Jordan (36.5%) and Poland (46%) (24–26). The results of a large meta-analysis that included cross-sectional data also supports lower serum testosterone levels in men with T2DM (11). However it is important to note that the reported prevalence may vary between studies depending on the assay method employed and the lower limit of normal range that is applied.

The prevalence of low serum testosterone has been shown to increase with age in the general population (4,9). The current study demonstrated a trend toward increasing prevalence of low serum TT with ageing which is in keeping with the available literature.

13.1.2 Sub-Saharan Africa

There is limited data on the prevalence of low serum testosterone in men with T2DM from SSA, with only a few studies from Nigeria and a single study each from Ghana and South Africa (16–20,27,28). Such studies report the prevalence of LSTT between 29.5% and 50%, which is similar to the 35.8% prevalence reported in this study (16,17,20,28). From the available studies only four reported the actual prevalence of LSTT among men with T2DM (16,17,20,28).

The only study that is comparable with the current study is the Ghanaian study that also measured serum FT. In that study Asare-Anane et al compared FT and TT levels in 105 men with T2DM and 105 nondiabetic men (17). Although mean serum FT was found to be lower in diabetic compared to control participants (0.2 ± 0.1 vs. 0.4 ± 0.1 nmol/l, $p<0.0001$), they did not report on the overall prevalence of LSFT in the diabetic group. The prevalence of LSTT <8 nmol/l and low TT between 8 and 12

nmol/l in diabetic men in Ghana was much higher, 35.2% and 20%, respectively; with the overall prevalence of TT <12 nmol/l of 55.2%. The higher prevalence cannot be explained on the basis of age, BMI and diabetes duration of the populations being studied. A possible reason is that in the Ghanaian study, recruitment of men was purely from a tertiary hospital based diabetes clinic whereas the current study included men from a tertiary hospital clinic as well as from a regional hospital based clinic. A higher prevalence has also been reported in another teaching hospital based study from Nigeria; the prevalence of LSTT < 8 nmol/l with a positive ADAM score was 29.5% and TT between 8 and 12 nmol/l with a positive ADAM score was 23% (28).

There are five published studies from Nigeria that measured TT with variable assay methods and definition for LSTT (18–20,27,28). Only three studies included a control group in the study design and reported consistent findings of a lower mean TT level among diabetic men compared to control subjects (18,19,27). The report by Akinloye et al (19) of extremely low TT levels in diabetic subjects compared with controls (3.1 ± 0.34 vs. 34 ± 3.9 nmol/l; p<0.0001) may have been accounted for by the inclusion of patients with newly diagnosed diabetes and concomitant hypertension into the study group. Ogbera et al, in a study on 203 Nigerian men with T2DM, reported a high prevalence (36%) of testosterone deficiency syndrome defined as a TT between 8 and 12 nmol/L with symptoms of hypogonadism or TT <8 nmol/L with or without symptoms of hypogonadism (20). Ubajaka et al described a significantly lower mean testosterone level in 125 diabetic men compared to 50 non-diabetic control subjects attending a university teaching hospital diabetes clinic in Nigeria (18). Fabian et al found significantly lower TT among 34 men with T2DM attending a university based diabetes clinic in Ibadan, Nigeria when compared to 53 control subjects (27.0 ± 3.0 vs 32.6 ± 2.5 nmol/l ; p=0.01) (27). In a cross-sectional survey of

200 men with T2DM attending a diabetes clinic at a teaching hospital in Nigeria, Ugwu et al found a 29.5% prevalence of LSTT (defined as <8 nmol/l). The prevalence of LSTT < 12nmol/l was 53% which is significantly higher than the 35.8% reported in the current study. Compared to the current study, the diabetic men in the study by Ugwu et al were similarly aged (57.9 vs. 57.5 yrs), had lower mean waist circumference (94.2 vs. 103 cm), shorter duration of diabetes (6.6 vs. 11.4 yrs) and these observations do not explain the difference in the prevalence of LSTT with the current study (28). However, all the patients in the study by Ugwu et al were sourced from a diabetes clinic at a teaching hospital whereas the patients in the current study included patients from a regional hospital based diabetes clinic.

13.1.3 South Africa

There is limited information on the prevalence of low serum testosterone in South African men with T2DM. To date, the only published report from South Africa is from an academic centre in Pretoria (16). Kemp et al evaluated 150 male subjects with diabetes attending a tertiary diabetes clinic at the Steve Biko Academic Hospital; of these 91% had T2DM (16). Diabetic men were screened for symptoms of androgen deficiency and only serum TT but not FT was measured. The prevalence of LSTT, defined as TT below the reference range of 9.9 nmol/l was very high (50%) compared to the current study (19%), despite the use of a higher cut-point of <12 nmol/l for the lower limit of TT in this study. Nevertheless, the overall findings of both studies are similar and reflect a high burden of low serum testosterone levels among men with T2DM. The higher burden of LSTT among men from the Pretoria study may possibly be related to differences in the background characteristics, including the mean duration of diabetes, age, waist circumference, cardiovascular disease prevalence and statin use. In the Pretoria study, participants were older (62 vs 57.5 years), had longer duration diabetes (15 vs 11.4 years), higher mean waist

circumference (112 vs 103 cm), higher burden of CVD (41% vs 18%) and a higher proportion were on statin therapy (93% vs 66%). Furthermore, there were striking differences in the ethnicity of the study groups with the Pretoria study including a majority white population (52%) whereas the Durban study included a majority African cohort (59%) with only a few white patients (2%). Indian patients formed a larger component of the study group in the current study (39%) while only contributing to 10% of the study population from Pretoria. Given these differences between the two studies, it is likely that the most important factors that explain the higher prevalence of LSTT in the Pretoria study is the older age and higher WC, which are well known associations of low serum testosterone in men.

13.1.4 Ethnicity

There was no significant difference in mean FT levels and prevalence of LSFT between African and Indian patients in this study and the number of white patients were too few to allow a comparison. Ethnic differences in serum testosterone levels have been described in the literature. A recent meta-analysis that included 15 studies, found that black men have a modest but significant 2.5-4.9% higher FT compared to white men; however this difference was not observed for TT (14). It has been suggested that ethnic variance may possibly be attributed to differences in steroid metabolism, genetics, geographic or environmental factors and body composition (13).

13.2 Androgen deficiency symptoms and the significance

In this study, androgen deficiency symptoms were assessed by direct enquiry of symptoms as well, with the self-administered AMS questionnaire. The overall prevalence of androgen deficiency symptoms was higher with the AMS score

(74.5%) compared to direct patient enquiry (69%), although this difference was not significant. There was no significant difference in the prevalence of an AMS score ≥27 in those with LSFT vs. NSFT (70.8% vs. 75.8%, p=ns). There was a low correlation between AMS scores and both FT and TT.

Screening men with T2DM for symptoms of androgen deficiency has been recommended by some organisations to justify or determine whether a patient would require measurement of serum testosterone levels (29). As noted in the introduction, a number of screening tools in the form of self-administered questionnaires have been developed but lack sufficient evidence to support their routine use in clinical practice. These tools have been developed for the general population but have not been specifically validated in diabetic populations. The few available studies in diabetic men have shown that the sensitivity of these questionnaires is high but they lack specificity; also, that they correlate poorly with serum testosterone (25,30,31). Despite this shortcoming, many physicians continue to use these questionnaires in the absence of an evidence-based alternative screening tool.

The value of using questionnaires for the detection of androgen deficiency symptoms and screening for testosterone deficiency in the African setting has not been adequately investigated and remains unclear. These tools have been developed with data derived from western populations and there is limited information on their accuracy and performance in patients from SSA countries. The only published data from SSA that investigated the reliability of a screening questionnaire was reported from Nigeria (32). This cross-sectional survey on 200 sub-Saharan African men with T2DM, evaluated the reliability of the Saint Louis University ADAM questionnaire and found high sensitivity (88.1%) but low specificity

(44.7%). The authors concluded that the ADAM questionnaire was an unreliable marker of testosterone deficiency in their population of men with T2DM. Moreover, they identified loss of libido as a reliable marker of testosterone deficiency with better accuracy than the ADAM questionnaire. In contrast, the current study did not identify any significant association between the symptoms of androgen deficiency on direct inquiry and low serum FT or low serum TT levels. Although decreased beard growth was found to be associated with LSFT on univariate analysis, this was not significant on multivariate analysis. In addition, neither the total AMS score nor its individual components were associated LSFT or LSTT.

Similar to the findings in the Nigerian study (32), the South African study from Pretoria also found a high prevalence of androgen deficiency symptoms (95%) using the ADAM questionnaire with high sensitivity (95%) and very low specificity (5%) (16). Sensitivity and specificity of the AMS questionnaire in the current study were not calculated due to the poor predictive probability of the score. The current study found the AMS questionnaire to be a poor screening tool and was a poor predictor of LSTT and LSFT. The lack of a significant difference in AMS scores or prevalence of high AMS score between those with LSFT and NSFT, further highlight the weakness of the questionnaire in our population.

Based on available data from SSA including two studies from South Africa, there appears to be no particular advantage to screening for androgen deficiency symptoms using a questionnaire rather than via direct enquiry. Therefore the value of both the AMS and ADAM questionnaires in SSA populations remain unresolved and requires further evaluation. Until there is strong evidence to validate their use, it

would be prudent to use the direct symptom enquiry viz. loss of libido, to screen for androgen deficiency symptoms.

13.3 Prevalence of Hypogonadism

The prevalence of hypogonadism in this study, defined as LSFT in conjunction with a positive AMS score, was 11.5%. From available literature, the prevalence of hypogonadism using similar criteria reported in western countries ranges from 20-42% (23,33). Although the prevalence of low serum testosterone among men with T2DM has been reported in several studies from SSA, to date only three studies from Nigeria have documented the prevalence of hypogonadism with rates ranging from 29.5 to 65.3%, higher than that observed in the current study (19,20,28). The likely explanation for this discrepancy is the differences in the definition of hypogonadism used in those studies. The study by Akinloye et al reported the highest rate of hypogonadism (65.3%) but the definition of hypogonadism that was applied was unclear (19). Ogbera et al (20) found a 36% prevalence of hypogonadism which was defined as TT < 12nmol/l with associated symptoms of hypogonadism which were obtained via direct enquiry and included erectile dysfunction, reduced libido, loss of concentration, irritability, hot flushes, reduced energy and depression. More recently, Ugwu et al (28) reported that the prevalence of overt hypogonadism (defined as TT < 8nmol/l together with a positive ADAM score) was 29.5% and that of possible hypogonadism (defined as TT 8-12 nmol/l together with a positive ADAM score) was 23%. If these criteria were applied to the current study then the corresponding prevalence of overt and possible hypogonadism using TT would be 7.4% and 18.9%, respectively. It was not possible make a comparison with the single South African study (16) because they did not report on the prevalence of hypogonadism in their cohort.

13.4 Aetiology of low serum testosterone levels: role of gonadotropins

It is now well recognized that the majority of diabetic men with low serum testosterone have normal or low levels of LH, compatible with hypogonadotropic hypogonadism (6,34). The prevalence of normal or low LH among men with low serum testosterone is reported to be 67-83% (23,25,35). However, few studies from SSA have reported on the role of gonadotropin levels in the aetiology of hypogonadism in diabetic men from this region.

The majority of patients (79.2%) with LSFT in the current study had either normal or low LH levels, with only 20.8% having elevated LH levels, compatible with reports from other continents (23,25,35). The corresponding rates for LSTT are also similar with 73.3% of men with TT <8 mmol/l having a low or normal LH level. Similar findings are reported from a Nigerian study (28) with 23.7% of hypogonadal men having an elevated FSH or LH level and 76.3% having either normal or low gonadotropin levels. No other data from SSA is available for comparison.

The current study found no differences in mean LH levels between LSFT and NSFT groups as well as between LSTT and NSTT groups. Limited data from SSA is available for comparison. Similar findings were reported by Ugwu et al who found no difference in mean LH levels in hypogonadal (TT<8 mmol/l) vs. eugonadal (TT ≥12 mmol/l) men with T2DM (28).

Two studies from SSA compared mean LH and FSH levels in patients with T2DM and control subjects with variable results (17,27). Fabian et al found no significant

differences in LH in Nigerians (27); by contrast, Asare-anane (17) found that in Ghanaians, when compared to control subjects, diabetic men had significantly higher mean LH levels, but not FSH levels. This latter finding is similar to the current study which also showed that diabetic men had higher LH levels compared to control subjects in univariate analysis, though FSH was not measured. It is important to note that mean LH levels were within the normal range in all these studies. The most likely explanation for the conflicting findings, is that the diabetic subjects in the Ghanaian and current study were significantly older than controls and hence more likely to have age related decreases in testicular function leading to relatively higher gonadotropin levels. This is also supported by results of multivariate analysis comparing control subjects to patients with T2DM in the current study which showed that age but not LH was a significant risk factor associated with TT.

13.5 Low testosterone and obesity

The association between testosterone and obesity which has been well documented (36), is confirmed by the findings of this study. A relationship between serum FT and TT with measures of adiposity such as waist circumference, hip circumference, BMI and metabolic syndrome was observed. Both FT and TT were inversely correlated with waist circumference and BMI. This has also been reported in earlier studies (37,38).

The significant risk factors associated with LSFT and LSTT in this study were waist circumference and BMI, compatible with reports from other studies (39,40). Waist circumference but not other measures of adiposity including hip circumference, weight, waist-to-hip ratio or BMI was significantly and independently associated with LSFT on multivariate analysis.

Mean BMI in this study was 29.8 kg/m^2 with 72% of men being overweight or obese, underscoring the importance of measuring FT levels in this high risk population of men with diabetes. The observed negative correlation between BMI and SHBG in this study confirms the findings in other studies (6). It has been postulated to be the reason for the low serum TT in men with diabetes. Although lower SHBG concentration related to higher obesity levels among diabetics may partly explain the lower TT, they do not account for the lower FT levels. Therefore, other factors such as elevated circulating adipokines or higher oestradiol levels which have been implicated in the pathogenesis of low serum testosterone in men with obesity, may also explain the effect in men with diabetes due to its association with obesity (41,42).

Although there is evidence that obesity and hypogonadism are related, it remains speculative as to which of the two conditions is the initiating factor. The prevailing hypothesis is that obesity precipitates hypogonadism and this is turn exacerbates obesity, leading inexorably to a self-perpetuating vicious circle. The hypogonadal-obesity cycle proposed by Cohen infers that accumulation of adipose tissue increases expression of aromatase, leading to higher oestradiol levels which inhibits gonadotropin releasing hormone (GnRH) and suppresses gonadotropin secretion. This ultimately leads to reduced gonadal testosterone secretion with consequent further deposition of adipose tissue (42). However, this theory has been challenged by a recent study which showed that total and free oestradiol levels are not elevated in diabetic men with hypogonadotropic hypogonadism (43). The most favoured current postulate is the hypogonadal-obesity-adipocytokine hypothesis (44) which implicates the pro-inflammatory adipokines elaborated by adipose tissue depots as the inciting agents in reducing GnRH signaling. Elevated circulating leptin levels may

also be partly responsible for the development of low testosterone in obese men (45). High leptin is believed to be consequent to presumed leptin resistance and contributes to altered hypothalamic GnRH signaling via kisspeptin, however this theory has not been well studied in humans and requires further evaluation (46).

As in this study, most other studies from SSA have investigated testosterone levels in men with diabetes and have found an association with some measure of adiposity. A few studies did not include anthropometric measures and one study showed no correlation between serum testosterone and the metabolic syndrome parameters (20). The consistent finding in the remainder of the studies was that low TT is associated with a higher waist circumference (16,19,28) or BMI (17,19) and that TT correlated inversely with BMI (17,19) or waist circumference (28). These findings are similar to that reported in studies from other parts of the world; although this evidence does not confirm causality, it supports the notion that obesity probably plays a central role in the pathogenesis of decreased serum testosterone levels in men with T2DM.

Notwithstanding, there are some patients that have low serum testosterone with a normal BMI; in this study, 14.8% of patients with a BMI < 25 kg/m^2 had LSFT. Although a normal BMI in these patients does not exclude the presence of central adiposity, it does suggest that other factors than adiposity may also be contributing to the development of hypogonadism. For example, insulin resistance though strongly associated with T2DM, can occur in normal weight subjects and has been shown in some studies to correlate with changes in testosterone (47). Animal models support the activity of insulin as a modulator of LH secretion, with lack of insulin action being associated with reduced LH and testosterone (48). Furthermore,

genetic factors may also be responsible to some degree, for the development of low serum testosterone in men with T2DM. Since obesity is strongly linked to the development of low testosterone in men with T2DM, it is of particular concern for SSA countries that have experienced an increase in obesity prevalence over the past decade. More disturbing is that South Africa has the dubious privilege of having the highest prevalence of obesity in SSA (49). If this trend continues, many more men will be at risk of developing low testosterone levels and the consequences thereof.

13.6 Relationship with age

The effects of ageing on testosterone levels among men in the general population has been extensively investigated and has consistently shown that testosterone progressively declines with advancing age. Landmark studies including the Massachusetts Male Ageing Study and Baltimore Longitudinal Study on Aging have also demonstrated that the prevalence of androgen deficiency increases with ageing (4,9). The MMAS study showed that the crude prevalence of testosterone deficiency in men aged over 40 years was 6% (4).

In the current study, serum FT was lower with advancing age. The lowest levels of mean FT and mean TT were noted in the oldest age category (>70 years). Age was also found to be a significant risk factor associated with LSFT. An inverse correlation was observed between age and FT. Therefore, the findings of this study regarding the association between LSFT and age are compatible with that reported in the general literature. In this study, the relationship with age was further highlighted by the absence of LSFT among all patients below the age of 40 years, irrespective of their symptom status. This may indicate that screening young patients below the age

of 40 years may not be worthwhile and perhaps unnecessary, even if they are symptomatic.

The findings in this study also compares well with published findings from other SSA countries. Two Nigerian studies reported on the relationship between age and serum testosterone in men with T2DM. Akinloye et al (19) showed that there was an inverse correlation between age and TT. Although a similar trend was reported in the Ghanaian study, this was not statistically significant (17). Ugwu et al demonstrated that age was associated with the presence of hypogonadism defined as LSTT with androgen deficiency symptoms (28).

In a South African cohort of the Transition and Health during Urbanisation of South Africans (THUSA) Study that investigated 364 male subjects between the ages of 20 and 82 years, testosterone levels also declined with age (50).

Since age is a probable factor that influences serum testosterone levels, it also has an important role to play in the selection of diabetic patients for screening of androgen deficiency, especially in resource limited settings where cost-effectiveness is paramount.

13.7 Relationship with glycaemia, lipids and metabolic syndrome

Hypogonadism in men with T2DM has been reported to be associated with morbidity which includes lower haematocrit, decreased bone mineral density and insulin resistance (51,52). Low serum testosterone has been linked to CVD and mortality in population based studies (53–55). Despite the evidence supporting the detrimental

effects of low testosterone on men with T2DM, the benefits of testosterone therapy on CVD have yet to be confirmed in randomized controlled clinical trials. Small studies have shown improvement in insulin resistance with testosterone replacement therapy (40). Whether low testosterone in men with T2DM impacts on glycaemic control remains unclear; moreover the effects of testosterone treatment on HbA_{1c} still remain controversial. A recent meta-analysis showed that testosterone replacement treatment did not improve glycaemic control (HbA_{1c}) in men with type 2 diabetes and/or metabolic syndrome (15).

In this study, no association was found between serum testosterone (FT and TT) and HbA_{1c} and there was no correlation between HbA_{1c} and serum testosterone. These findings support those from western countries as well as other studies from SSA (6,16,17,28,40).

With respect to serum lipids, no association was noted between serum testosterone (FT and TT) and total cholesterol, LDL-cholesterol, HDL-cholesterol and total triglycerides. However, the finding of a weak inverse correlation between serum TT and serum total triglycerides, is similar to two other studies from SSA and requires further evaluation (17,19).

The number of components of the metabolic syndrome also correlated with serum FT and TT. This relationship between metabolic syndrome and low serum testosterone in men with T2DM was also observed with one other study from Nigeria (19) and has been well described in the literature (56–58). Moreover, in this study waist circumference was the best measure of adiposity for predicting LSFT. Therefore, it seems that the strongest association between low serum testosterone

and features of the metabolic syndrome occurs with waist circumference which is a surrogate for visceral adiposity.

In this study, no association was found between low serum testosterone and a history of CVD. However, the study was not designed to investigate this relationship and the numbers of patients too low to make any conclusions.

13.8 Evidence for testosterone screening recommendations

Current international guidelines recommend screening men with T2DM for low testosterone (5). There are no national South African policy documents to guide healthcare practitioners managing men with T2DM regarding screening this high risk population for androgen deficiency. However, the 2017 South African guideline document for the management of T2DM published recently by the Society for Endocrinology Metabolism and Diabetes of South Africa (SEMDSA), states that screening for low serum testosterone levels among men with T2DM and symptoms of hypogonadism is mandatory (29). Taken collectively, the available evidence from SSA countries including data from two South African centres, indicate a high prevalence of low serum testosterone and therefore deserve mention in future guidelines from these regions. However, given that most SSA countries are low-income regions, it is imperative that the cost-benefit ratio of screening be taken into consideration before implementation of any recommendations.

What appears obvious from the two South African studies is that the majority of men with T2DM suffer from symptoms of androgen deficiency. However, the ADAM questionnaire used in the Pretoria study and the AMS questionnaire employed in the

Durban (current) study were found to be inaccurate in detecting low serum testosterone because of their low specificity or poor predictive capability. This highlights the need for studies to validate the use of these questionnaires in screening for androgen deficiency among men with T2DM. In the interim, if South African men are to be questioned about symptoms of androgen deficiency, then asking about erectile dysfunction and loss of libido must be considered first, since these are the most frequently reported symptoms in the two South African studies.

More national data are required before recommendations can be made regarding a universal screening policy for all South African men with diabetes. However, from the available evidence it appears that screening for androgen deficiency could be justified in this high risk population. Men most likely to benefit from screening for low testosterone would be older and overweight with predominant central adiposity. The findings from this study do not support screening men younger than 40 years of age. Although the exact age cutoff to implement routine screening of serum testosterone levels in men with T2DM is unclear, the value of routinely measuring serum testosterone in all men over the age of 60 years and those with central obesity merit further investigation.

13.9 Study Strengths

The study was conducted at two centres and included men from the outpatient diabetes clinic at a district level and tertiary hospital. All the available research from SSA have been single centre studies, usually from an academic institute. Therefore, the findings of this study are significant and more representative of the typical diabetic male patient managed at an outpatient diabetes clinic. This study was able to determine the prevalence of both LSFT and LSTT. Although FT provides a more

accurate reflection of testosterone status than TT, only one other study from SSA data reported the prevalence of LSFT in men with T2DM. A detailed history was obtained from all study subjects, allowing the potential effects of pharmacotherapy and smoking on serum testosterone to be considered. This is only the second study from South Africa to report on the prevalence of low serum testosterone in men with T2DM. Few studies have evaluated the value of the AMS questionnaire in men with T2DM and the data from this study adds to the literature. No other study from SSA has used the AMS score.

13.10 Study Limitations

One of the major limitations was the cross-sectional study design, which does not allow for examination of predictive risk factors, only risk association. A prospective study may have been able to clarify the association of low serum testosterone with CVD and mortality in South African men with T2DM. The sample size could have been larger in this study. This research did not include investigation of the effects of testosterone treatment in the study design. This study did not evaluate the possible effect of socio-economic status on serum testosterone levels. The findings of this study may not be extrapolated to all provinces, since it was not representative of all South African men and did not recruit enough white and coloured (mixed-race) men.

13.11 Conclusions

In this group of predominantly African and Indian men with type 2 diabetes mellitus (T2DM) from KwaZulu-Natal, the prevalence of low serum total and free-testosterone was high, and is in accordance with that reported in the literature. Low serum total testosterone (LSTT) was associated with a higher body mass index and older age. Waist circumference was a significant risk factor associated with low serum free-testosterone (LSFT) among men with diabetes. LSFT correlated with age, body mass index, waist circumference, waist-to-hip ratio and the number of components of the metabolic syndrome. There was no correlation between serum testosterone and HbA_{1c}. Men with T2DM had lower serum free-testosterone levels than control subjects after correction for age and body mass index.

Using the Ageing Male's Symptom Scale (AMS) questionnaire, there was a high prevalence of androgen deficiency symptoms which was noted across all age groups. However, low serum testosterone was not associated with symptoms of androgen deficiency in this study. The AMS questionnaire performed poorly in this population and was found to be a poor predictor of low serum total and free-testosterone. The poor correlation between serum testosterone (total and free) and the AMS score also underscores the weakness of the questionnaire in this population. Furthermore, there was no significant difference between the prevalence of symptoms on direct enquiry compared to the AMS questionnaire. The most frequent symptoms on direct enquiry were erectile dysfunction and loss of libido.

The aetiology of low serum testosterone in this study is thought to be multifactorial. A small percentage of patients had elevated luteinizing hormone levels compatible with a primary testicular aberration. In keeping with the prevailing hypothesis however, the most likely cause of androgen deficiency was thought to be due to a defect in the hypothalamic-pituitary axis, since the majority of patients had a low or normal luteinizing hormone level.

Given the available data from South Africa demonstrating a high prevalence of low serum testosterone levels in men with T2DM and that suitable therapy is available, screening is justifiable in this population. However, more research is required locally to conduct a cost-benefit analysis before screening can be recommended.

13.12 Implications for Practice

Healthcare professionals must have a greater awareness of possible androgen deficiency in men with T2DM. Symptoms of androgen deficiency can be sought by direct patient enquiry or via a standardized questionnaire. Screening for low serum testosterone should be considered in this population if the resources are available. Selecting patients for screening based on risk factors such as men who are older or obese, in particular those with central adiposity, may increase the yield. Routine screening below the age 40 years in the absence of risk factors is not advised.

13.13 Implications for Research

More research is required from other provinces in South Africa to confirm the findings of this study and allow a better understanding of the underlying risk factors that may be associated with low serum testosterone. This will assist in developing more appropriate local guidelines for screening in the South African context. Specifically, more data is required to determine the most appropriate clinical circumstances that should prompt screening. With the burgeoning cost of health-care in South Africa, it is imperative that the cost of screening be justified. Therefore research into the cost-benefit ratio of screening is required to determine its value in resource poor settings. In addition there is also a dire need for more data from other SSA countries. The validity of standardized questionnaires in screening for androgen deficiency also warrants further investigation. It is hoped that the findings of this

research would stimulate a longitudinal study in South Africa to determine if low serum testosterone levels are associated with cardiovascular disease or mortality and whether appropriate therapy with testosterone can improve glycaemic control and/or attenuate cardiovascular disease risk.

APPENDICES

Appendix 1

PREVALENCE OF LOW TESTOSTERONE LEVELS IN T2DM STUDY							
Data Sheet/Questionaire (Interviewer Administered)							
Subject Source :	IALCH	PCHC		**Date of completion :**			
				Study No:			
DEMOGRAPHICS							
Ethnicity				Endo Number			
Date of Birth				Duration of diabetes			
Age	Current	Diagnosis		Using any insulin therapy	YES	NO	
SYMPTOMS				**PAST MEDICAL HISTORY**			
Erectile Dysfunction	YES	NO		Testosterone deficiency	YES	NO	
Loss of Libido	YES	NO		Past treatment with testosterone	YES	NO	
Poor Beard growth	YES	NO		Past exposure to anabolic steroids	YES	NO	
Infertility	YES	NO		Radiotherapy	YES	NO	
				Chemotherapy	YES	NO	
DIABETES COMPLICATIONS				Testicular torsion	YES	NO	
Any Retinopathy	YES	NO		Orchidopexy or orchidectomy	YES	NO	
Microalbumiuria	YES	NO		Previous orchitis eg mumps or TB	YES	NO	
Proteinuria	YES	NO		Trauma to testes eg crush injury	YES	NO	
Ischaemic Heart Dx	YES	NO		History of Head trauma	YES	NO	
Peripheral Vasc Dx	YES	NO		Neurosurgery to pituitary fossa	YES	NO	
CVA or TIA	YES	NO		HIV / AIDS	YES	NO	
CHRONIC DISEASE and DRUG HISTORY							
Hypertension	YES	NO		Specify drug(s):			
Depression	YES	NO		Specify drug(s):			
Parkinsons	YES	NO		Specify drug(s)			
Recreational drugs	YES	NO		Specify: eg. ROH, cocaine, dagga			
Other chronic drugs	YES	NO		Specify:			
CLINICAL							
(a) Height (cm)				(g) Gynaecomastia	YES	NO	
(b) Weight (kg)				(h) Decreased beard growth	YES	NO	
(c) Body mass index							
(d) Waist circumferen.				(i) Testicular volume (mls)			
(e) Hip circumference				(use prader orchidometer)			
(f) Waist:Hip ratio				(j) Blood pressure (mmHg)			
BLOOD RESULTS							
Total Testosterone				Total Protein			
FSH				Albumin			
LH				Alkaline phosphatase			
Prolactin				G-glutamyl transferase			
Total cholesterol				Alanine transaminase			
Triglycerides				Aspartate Transaminase			
HDL cholesterol				Haemoglobin			
LDL cholesterol				Haematocrit			
				HbA1c			
free Testosterone				Fructosamine			

Appendix 2: AMS Questionnaire (English)

	HOSPITAL No: _____		**STUDY No:** _____				
	Ageing Male's Symptom Questionaire						
	Which of the following symptoms apply to you ? Please mark the appropriate box.						
	SYMPTOMS		**None**	**Mild**	**Moderate**	**Severe**	**Extremely Severe**
		Score	**1**	**2**	**3**	**4**	**5**
1	**Decline in your feeling of general well-being** (general state of health, subjective feeling)						
2	**Joint pain and muscular ache** (lower back pain, joint pain, pain in a limb, general back ache)						
3	**Excessive sweating** (unexpected/sudden episodes of sweating, hot flushes independent of strain)						
4	**Sleep problems** (difficulty in falling asleep, difficulty in sleeping through, waking up early and feeling tired, poor sleep, sleeplessness)						
5	**Increased need for sleep, often feeling tired**						
6	**Irritability** easily upset about little things, moody)						
7	**Nervousness** (inner tension, restlessness, feeling fidgety)						
8	**Anxiety** (feeling panicky)						
9	**Physical exhaustion / lacking vitality** (general decrease in performance, reduced activity, lacking interest in leisure activities, feeling of getting less done, achieving less, force oneself to undertake activities)						
10	**Decrease in muscular strength** (feeling of weakness)						
11	**Depressive mood** (feeling down, sad, on the verge of tears, lack of drive, mood swings, feeling nothing is of any use)						
12	**Feeling that you have passed your peak**						
13	**Feeling burnt out, having hit rock-bottom**						
14	**Decrease in beard growth**						
15	**Decrease in ability/frequency to perform sexually**						
16	**Decrease in the number of morning erections**						
17	**Decrease in sexual desire/libido** (lacking pleasure in sex, lacking desire for sexual intercourse)						

Appendix 3: AMS Questionnaire (Zulu)

Imibuzo ngezimpawu ezikhombisa ukukhula (ubudala) komuntu wesilisa AMS Questionaire					
Phakathi kwalezi zimpawu ezilandelayo yiziphi onazo njengamanje? Uyacelwa ukuba ukhethe ibhokisi elilodwa ngophawu onalo/izinkomba onazo. Uma zingengo lezozimpawu emzimbeni wakho bhala “akwenzeki”					
Izimpawu:	**Akwenzeki**	**Kancane**	**Kakhudlwana**	**Kakhulu**	**Kakhulu ngokwedlulele**
Umphumela =	1	2	3	4	5
1. **Ukungazizwa kahle (**ngokwempilo)					
2. **Ubuhlungu kwamajoyinti nezicubu zomzimba** (ubuhlungu beqolo, kwamajoyinti kwezimbambo, ubuhlungu komhlane					
3. **Ukujuluka ngokweqile** (okujuluka okuqhamuka ungalindele, ukuba nezikhawu zokushiselwa kakhulu)					
4. **Inkinga yokungalali** (ukungafiki kobuthongo, ukungakwazi ukulala ubusuku bonke, ukusheshe uvuke uzizwe ukhathele, ukulala kabi, ukungalali nhlobo)					
5. **Ukuzizwa udinga ukulala, ukuhlezi uzizwa ukhathele**					
6. **Ukubanenhliziyo encane** (ube nolaka, ucasulwe yizinto ezincane, ungaqondakali - uhleke manje usheshe udinwe					
7. **Ukwesaba** (ukungakhululeki emoyeni, ukungabi nasinqe, uxhamazele)					
8. **Ukutatazela** (uzizwe wesaba)					
9. **Uzizwe ukhathele / ungenawo umdlandla** (ukwehla kothando lokwenza izinto ezithile ezisebenzisa umzimba, ungabi nothando lokuvocavoca umzimba, ukwenqena, ufise ukwenza izinto ezincane, ukuziphoqa uma kufanele wenze izinto ezizosebenzisa umzimba)					
10. **Ukwehla kwezicubu / amamasela** (uzizwe ungenawo amandla)					
11. **Ubenomuzwa wokungakhululeki emqondweni** (uzizwe uphansi, uphatheke kabi, ufisa ukukhala, ungenawo umdlandla, ube neconsi, ube nomuzwa wokuthi ayikho into ewusizo empilweni)					
12. **Uzwe ukuthi awusenawo umdlandla owawunawo esikhathini esedlule**					
13. **Uzwe ukuthi sewenele yizinto ezenzekayo, awusakwazi ukubekezela**					
14. **Ukungakhuli kwentshebe**					
15. **Ukwehla kwezinga lokukwazi ukwenza ucansi/ ukwehla kwezikhathi owenza ngazo ucansi**					
16. **Ukwehla kwamanani okuvuka kwenduku ekuseni**					
17. **Ukwehla kwezinga lokufisa ukwenza ucansi / lokuhalela ucansi** (ukungajabuli uma wenza ucansi,ukungafisi ukwenza ucansi)					

Appendix 4: Information Leaflet

Information Sheet:Testosterone Deficiency in Type 2 Diabetes Study

Date: 3rd April 2013

Good day dear patient,

My name is Dr Imran M Paruk from the Department of Diabetes and Endocrinology at Inkosi Albert Luthuli Hospital. Telephone number : 031-2401299. Email address : paruki@ukzn.ac.za

You are being invited to participate in a study that involves research about identifying low testosterone levels among patients with type 2 diabetes. Low testosterone levels may be associated with sexual problems like low libido and erectile dysfunction. The purpose of this research is to determine how common this condition is among patients with type 2 diabetes. The study is expected to enroll about 150 or more patients from the adult diabetes clinics at Inkosi Albert Luthuli Hospital and the Diabetes Clinic at Phoenix Community Health Center. It will involve the following procedures : completion of a questionnaire and a clinical assessment by a doctor followed by blood tests. If you agree to participate, these procedures will be done during your routine clinic visit.

Participation in the study may have the following discomforts : Blood will need to be taken once only. The volume required would be about 4 teaspoons. As a study participant, you may benefit directly from this study because should you be identified as having low testosterone levels, it is a treatable disorder that may improve your quality of life.

This study has been ethically reviewed and approved by the UKZN Biomedical research Ethics Committee. In the event of any problems or concerns/questions you may contact the researcher at the following telephone number: 031-2401299 or the UKZN Biomedical Research Ethics Committee, contact details as follows:

BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Research Office, Westville Campus
Govan Mbeki Building
Private Bag X 54001, Durban, 4000, KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769
Fax: 27 31 2604609
Email: BREC@ukzn.ac.za

Remember that participation in this research is completely voluntary and that you are free to withdraw participation at any point. No explanation is necessary when informing us of your decision. In the event of refusal/withdrawal of participation, you will not incur any penalty or loss of treatment or other benefit to which you are normally entitled. Should you wish to withdraw your participation in the study, kindly contact Dr Imran M. Paruk at the Diabetes clinic, Inkosi Albert Luthuli Hospital and inform him of your decision (Telephone 031-2401299).

Should you participate in the study, all the tests will be undertaken without any cost to you. All information obtained during the course of this study including clinical and blood results will be treated as confidential. Primary data will be kept for 10 years. However, the information obtained during the study will be indefinitely available to all doctors who have access to your file on the computer network at Inkosi Albert Luthuli Hospital.

CONSENT DOCUMENT

CONSENT TO PARTICIPATE IN A STUDY

I_____have been informed about the study entitled "**Prevalence of low testosterone levels in a cohort of South African men with type 2 diabetes attending an outpatient diabetes clinic**" by Dr Imran M. Paruk.

I understand the purpose and procedures of the study. I have been given an opportunity to answer questions about the study and have had answers to my satisfaction.

I declare that my participation in this study is entirely voluntary and that I may withdraw at any time without affecting any treatment or care that I would usually be entitled to.

If I have any further questions/concerns or queries related to the study I understand that I may contact the researcher at the Diabetes Clinic in Inkosi Albert Luthuli Hospital. (Telephone number : 031-2401299).

If I have any questions or concerns about my rights as a study participant, or if I am concerned about an aspect of the study or the researchers then I may contact: **Biomedical Research Office** at the Nelson R Mandela School of Medicine at **031-260 4769**

_____ _____

Signature of Participant **Date**

_____ _____

Signature of Witness **Date**

_____ _____

Signature of Translator **Date**

(Where applicable)

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CONTRIBUTORS AND AUTHORSHIP

Name	Department	Contribution	Author or Acknowledgement
Dr I.M Paruk	Diabetes and Endocrinology	Investigator	Author
Prof A.A Motala	Diabetes and Endocrinology	Supervisor	Author
Dr F.J Pirie	Diabetes and Endocrinology	Co-supervisor	Author

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