ESTIMATION OF SERUM TESTOSTERONE LEVEL AND ITS CO-RELATION WITH HEMATOCRIT IN TYPE 2 DIABETES MALE PATIENTS - ONE YEAR HOSPITAL BASED PROSPECTIVE CASE-CONTROL STUDY IN KLES DR PRABHAKAR KORE CHARITABLE HOSPITAL & MRC, BELGAUM

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ENDORSEMENT

This is to certify that the dissertation entitled **Estimation of Serum Testosterone level and its co-relation with Hematocrit in Type 2 diabetes male patients- One year hospital based prospective case-control study in KLES Dr Prabhakar Kore Charitable Hospital & MRC, Belgaum** is a bonafide research work done by **(REG NO. BG0114007)**

Dr. Rekha S Patil MD
Professor and Head,
Department of Medicine,
J. N. Medical College,
Nehru Nagar, Belagavi – 10

Date:
Place: Belagavi.

Dr. N. S. Mahantshetti MD
Principal,
J. N. Medical College,
Nehru Nagar, Belagavi – 10

Date:
Place: Belagavi.

ABBREVATIONS

Type 2 DM	–	Type 2 Diabetes Mellitus
SHBG	–	Sex hormone binding globulin
ED	–	Erectile dysfunction
AR	–	Androgen receptor
FSH	–	Follicle stimulating hormone
LH	–	Luteinising hormone
GnRH	–	Gonadotropin releasing hormone
TNF	–	Tumour necrosis factor
IL	–	Interleukin
EPO	–	Erythrpoietin
Hb	–	Hemoglobin
ADA	–	American Diabetes Association
RBS	–	Random blood sugar
FBS	–	Fasting blood sugar
PPBS	–	Post prandial blood sugar
Sr	–	serum
TIBC	–	Total iron binding capacity
HbA1c	–	Glycated hemoglobin
ELISA	–	Enzyme Linked Immunosorbent Assay
BMI	–	Body Mass Index

ABSTRACT

INTRODUCTION:

Diabetes mellitus is heterogenous group of metabolic disorders. Hypogonadism is a clinical condition comprising both clinical and biochemical parameters of testosterone deficiency. Elder age and obesity both are linked with type 2 diabetes and both diminutions testosterone levels. Anaemia is frequently found in patients with diabetes, in whom it is associated with increased morbidity and mortality. Low testosterone levels are also common in men with type 2 diabetes.

AIMS AND OBJECTIVES:

AIMS: Estimation of Serum Testosterone level and its co-relation with Hematocrit in Type 2 diabetes male patients.

OBJECTIVES:

1. To estimate the serum testosterone in type 2 diabetes mellitus.
2. To estimate anemia and hematocrit in type 2 diabetes mellitus.

MATERIALS AND METHODS:

A Prospective Case Control study, Patients presenting to departments of Internal medicine both inpatients and outpatients at KLES Dr Prabhakar Kore Charitable Hospital & MRC, Belgaum fulfilling the inclusion criteria. 75 diabetic cases and 75 age and BMI matched non diabetic controls were taken for study.

RESULTS:

In our study, we found 72% of diabetic cases had low tesotosterone, mean hematocrit value was 41.81%, in both cases and controls. Normocytic normochromic anemia was common finding. There was significant corelation between testosterone and hematocrit values, with p=0.000042. There was also significant corelation between duration of diabetes, BMI, regularity of treatment for diabetes with serum testosterone levels.

CONCLUSION:

These findings suggest that testosterone deficiency may contribute to the increased frequency of anaemia in men with type 2 diabetes. And low testosterone can be a predictor of insulin resistance and metabolic syndrome, further studies are needed for confirmation of low testosterone with coronary artery disease.

KEYWORDS:

Serum testosterone,type 2 diabetes mellitus, hematocrit, anemia, insulin resistance.

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INTRODUCTION

Diabetes mellitus is heterogenous group of metabolic disorders characterised by chronic hyperglycemia with disturbances of fat, protein and mainly carbohydrate metabolism resulting from defects in insulin secretion or action or both.[1] several population studies shows increased incidence of hypogonadism in type 2DM.

Hypogonadism is a clinical condition comprising both clinical and biochemical parameters of testosterone deficiency. Major symptoms of hypogonadism are reduced or loss of libido, reduced strength and mood changes.[2]

Erectile dysfunction is common in diabetic men and the etiology may be vascular disease ,autonomic neuropathy, hypogonadism ,or a combination.While obesity contribute to the association of type 2 diabetes with hypogonadism, the association is not entirely dependent on obesity. It is possible that the association is mediated via insulin resistance, while all obese men are not insulin resistant[3].

It has been suggested that augmented aromatase activity in the adipose tissue will lead to a more degree of transformation from testosterone to estradiol and that the hypothalamus hypophseal axis[5] is inhibited by excess estradiol. On the other hand it has also been revealed that the removal of the insulin receptor gene in neurons of mice prime to hypogonadotropic hypogonadism. Thus the insulin resistant state in the hypothylamus leads to hypogonadotropic hypogonadism[4]. These observations have a number of clinical implications, subsequently low testosterone concentrations may contribute to weakening of sexual function, reduced libido and erectile dysfunction. Also the lack of testosterone during these vital years may lead to reduced peak bone mass and the lack of growth or loss of skeletal muscle[7].In addition these patients may

progress increased adiposity and therefore may become more insulin resistant .Clearly patients with type 2 diabetes specially if they are obese, need more focus attention and organized investigations. This is even more appropriate to younger type 2 diabetic patients.

Elder age and obesity both are linked with type 2 diabetes and both diminutions testosterone levels. Sex hormone- binding globulin (SHBG), the major serum carrier protein for testosterone also may have an effect. SHBG levels fall with obesity and rises with aging. Some studies shows lower SHBG level in type 2 diabetes patients. Most of the differences in testosterone levels between diabetes and non diabetes patients may be due to reduced SHBG, rather than reduced testosterone production. Though free testosterone levels fall with increasing age and obesity, rendering many type 2 diabetes patients testosterone deficient[5].

Testosterone replacement may increase insulin sensitivity in hypogonadal overweight men by varying body composition and by inhibition of lipoprotein lipase activity resultant condensed triglyceride uptake and accelerated triglyceride release from abdominal adipose tissue. The reduction in adipose tissue may also decline circulating free fatty acids, resulting in an advance in insulin sensitivity.

Testosterone treatment decreases insulin resistance and increases glycaemic control in hypo gonadal men with type 2 diabetes. The UK Prospective Diabetes Study (UKPDS) reported that a decrease in HbA1c in type 2 diabetic patients was associated with reduced micro-vascular problems as well as myocardial infarction. Males with coronary artery disease do have significantly lesser levels of bio-available, Free testosterone and testosterone therapy can recover ischaemia in men with chronic stable angina. Thus androgen replacement therapy in hypo gonadal type 2 diabetic

men could potentially improve glycaemic control and reduce microvascular and cardiovascular proceedings in these patients.

With this contextual we plan to assess our patients with type 2 diabetes mellitus for the presence of hypogonadism and treat them for their improved health and to avoid them from developing the complications of hypogonadism.

OBJECTIVE OF THE STUDY

- Estimation of Serum Testosterone level and its co-relation with Hematocrit in Type 2 diabetes male patients.
- To estimate the serum testosterone in type 2 diabetes mellitus.
- To estimate anemia and hematocrit in type 2 diabetes mellitus.

REVIEW OF LITERATURE

Diabetes mellitus is a multifactorial disease which is characterised by hyperglycaemia, dyslipidaemia, involves various organ systems, and results in various long-term complications. Several studies have suggested that menwith low testosterone levels are at a greater risk of developing type 2 diabetes mellitus, and that low testosterone levels may even predict the onset of

diabetes[1,2,3] .

Various studies have highlighted the high prevalence of low total and free testosterone (late-onset hypogonadism or testosterone deficiency syndrome) in men with type 2 diabetes and demonstrated links with visceral adiposity, insulinresistance, HbA1c, and symptoms of hypogonadism such as erectile dysfunction(ED) and low sexual desire.[4,5] Insulin resistance and visceral obesity areimportant features of type 2 diabetes[6] and are established markers of cardiovascular risk. An inverse relationship has been established between testosterone levels and insulin concentration in healthy men.[7] Low testosterone has been found to predict insulin resistance and later appearance of metabolic syndrome and type 2 diabetes, as well as a significant increase in all-causeand cardiovascular mortality in long-term studies.[7,8].

Testosterone stimulates erythropoiesis via production of haematopoietic growth factors and possible by iron bioavailability improvement[9]. Testosterone deficiency contributed to an increased frequency of anemia in men with type 2 diabetes mellitus and low testosterone and chronic inflammation contributed to mild anemia in type 2 diabetic men[10,11],have lower testosterone levels comparedto men

without a history of diabetes, and low testosterone are now being recognised as an independent risk factors for obesity, metabolic syndrome and type 2 diabetes[12,13].

Hemoglobin and hematocrit increase significantly under the action of testosterone, and erythrocytosis is the most frequent adverse event associated with testosterone therapy[14,15]. The mechanisms by which testosterone stimulates erythropoiesis are poorly understood, both testosterone dose and mode of delivery affect the magnitude of hematocrit elevation[16].

The Endocrine Society Guideline on Androgen Deficiency Syndromes in Men recommends hematocrit monitoring 3 months after initiation oftestosterone therapy and annually thereafter[17].

Androgens play a crucial role in the development and maintenance of male reproductive and sexual functions, body composition, bone health, and behaviour. Low levels of circulating androgens in utero can cause disturbances in male sexual development, resulting in congenital abnormalities of the male reproductive tract. Later in life, this may cause reduced fertility, sexual dysfunction[18], decreased muscle formation and bone mineralisation, disturbances of fat metabolism, and cognitive dysfunction. Testosterone levels decrease slightly as a process of ageing: signs and symptoms caused by this decline can be considered a normal part of ageing[19].

However, low testosterone levels are also associated with obesity and several chronic diseases, and some symptomatic patients may benefit from testosterone treatment[20].

Role of testosterone for male reproductive health :

Androgens, which are produced by the testis and by the adrenal glands, play a pivotal role in male reproductive and sexual function. Androgens are crucial for the development of male reproductive organs, such as the epididymis, vas deferens, seminal vesicle, prostate and penis[21]. In addition, androgens are needed for puberty, male fertility, male sexual function, muscle formation, body composition, bone mineralisation, fat metabolism, and cognitive functions[22].

Physiology:

Male sexual development starts between the 7th and 12th week of gestation. The undifferentiated gonads develop into a foetal testis through expression of multiple genes located on the short arm of the Ychromosome, including the sex-determining region of the Y chromosome (SRY gene complex) and the SOXgenes on chromosome 17[23].

The foetal testis produces three hormones: testosterone, insulin-like peptide 3(INSL3) and anti-Müllerian hormone (AMH). Testosterone is needed for the stabilisation of the Wolffian ducts,resulting in formation of the epididymis, vas deferens and seminal vesicle.[24]

AMH activity results in regression ofthe Müllerian ducts (Figure 1). INSL3 and AMH regulate testicular descent.Under the influence of intratesticular testosterone, the number of gonocytes per tubule increasesthreefold during the foetal period [25]

In addition, testosterone is needed for development of the prostate,penis and scrotum. However, in these organs testosterone is converted into the more potent metabolite 5a-dihydrotestosterone (DHT) by the enzyme 5a-reductase. Testosterone and DHT are required for penile growth, both activating the androgen receptor [26].

Intratesticular testosterone is needed to maintain the spermatogenic process and to inhibit germ cell apoptosis [27]. The seminiferous tubules of the testes are exposed to concentrations of testosterone 25-100 times greater than circulating levels. Suppression of gonadotrophins (e.g. through excessive testosterone abuse) results in a reduced number of spermatozoa in the ejaculate and hypospermatogenesis[28]. Complete inhibition of intratesticular testosterone results in full cessation of meiosis up to the level of round spermatids [27,28]

Testosterone does not appear to act directly on the germ cells, but functions through the Sertoli cells by expression of the androgen receptor (AR) and influencing the seminiferous tubular microenvironment [29].

Testosterone can also be metabolised into oestradiol by aromatase, present in fat tissue, the prostate, the testes and bone. Oestradiol is essential for bone mineralisation, also in men [30].

The production of testosterone is controlled in the foetus by placental choriongonadotropin (hCG) and after birth by luteinising hormone (LH) from the pituitary gland. Immediately after birth, serum testosterone levels reach adult concentrations over several months (minipuberty). Thereafter and until puberty, testosterone levels are low, thus preventing male virilisation. Puberty starts with the production of gonadotrophins, initiated by gonadotrophin releasing hormone (GnRH)

secretion from the hypothalamus and resulting in testosterone production, male sexual characteristics and spermatogenesis [31].

The androgen receptor (AR) :

Testosterone exerts its action through the AR, located in the cytoplasm and nucleus of target cells. During the foetal period, testosterone increases the number of ARs by increasing the number of cells with the AR, but also by increasing the number of ARs in each individual cell[32].

The AR gene is located on the X chromosome (Xq 11-12) defects and mutations in the AR gene can result in male sexual mal development, which may cause testicular feminisation or low virilisation (i.e. disorder of sexual development [DSD]).

Less severe mutations in the AR gene may cause mild forms of androgen resistance and male infertility[33]. In exon 1 of the gene, the transactivation domain consists of a trinucleotide tract (cytosine-adenine-guanine [CAG-repeats]) of variable length. Androgen sensitivity may be influenced by the length of the CAG repeats in exon 1 of the AR gene[33].

The AR CAG repeat length is inversely correlated with serum total and bioavailable testosterone and oestradiol in men. Shorter repeats have been associated with an increased risk for prostate disease, and longer repeats with reduced androgen action in several tissues[34]. CAG repeat number may influence androgenic phenotypical effects, even in case of normal testosterone levels.[34]

Conclusion :

Testosterone is essential for normal male development.

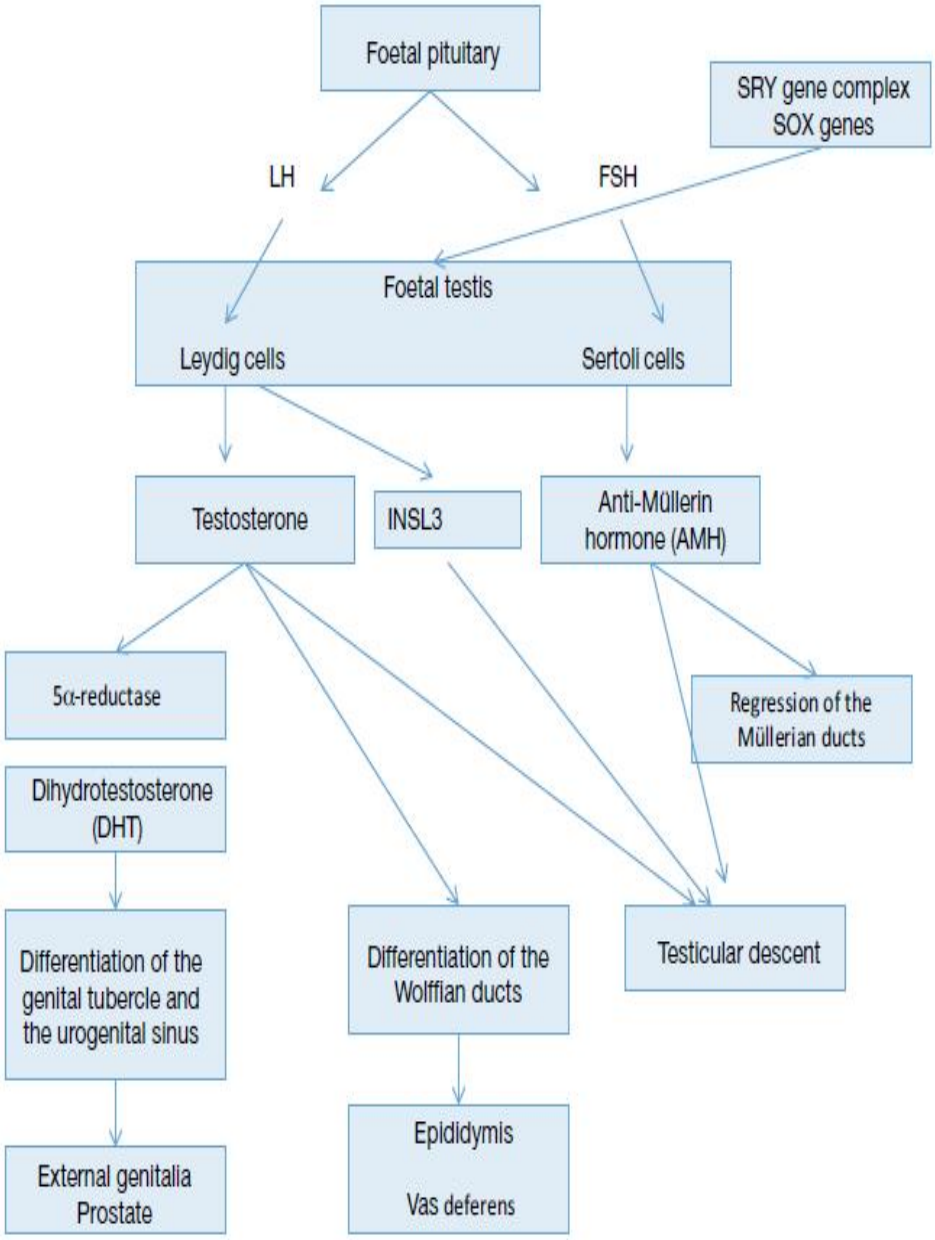
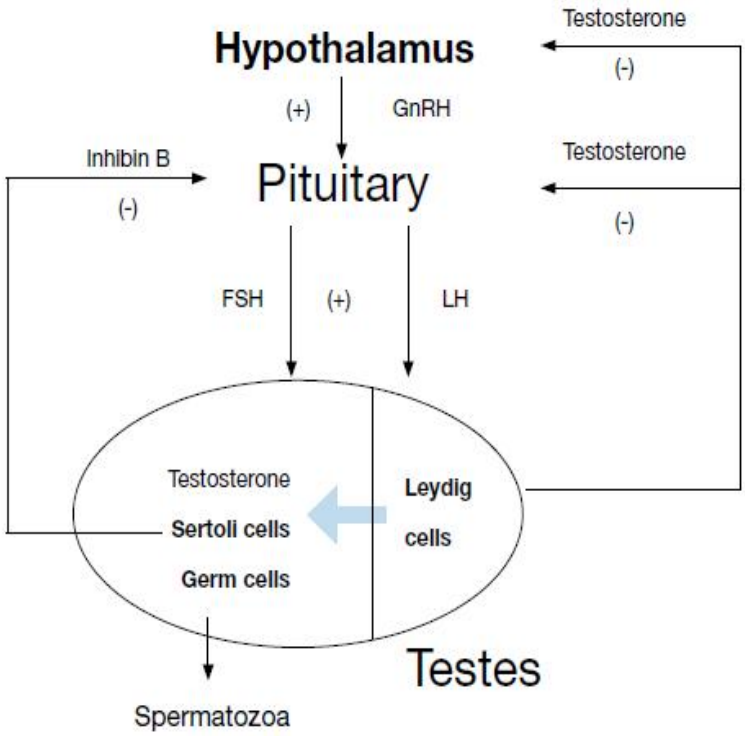


FIGURE 1: DEVELOPMENT OF THE MALE REPRODUCTIVE SYSTEM[35]

FSH = follicle-stimulating hormone; LH = luteinising hormone; SRY = sex determining region of the Y chromosome; INSL3= insulin-like peptide 3.

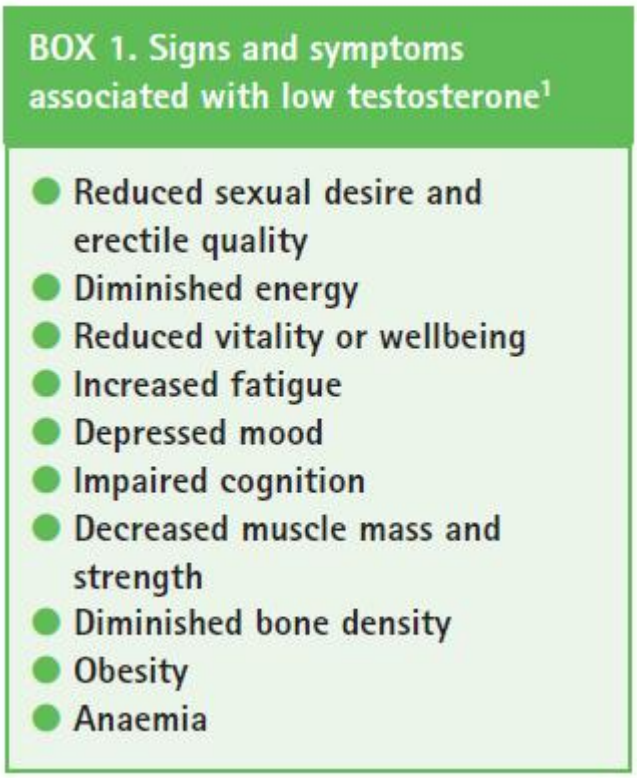
FIGURE 2: THE HYPOTHALAMIC-PITUITARY-TESTES AXIS



FSH = follicle-stimulating hormone; GnRH = Gonadotrophin-releasing hormone; LH = luteinising hormone

The HPA axis has a central role in regulating many homeostatic systems in the body, including the metabolic system, cardiovascular system, immune system, reproductive system and central nervous system. The HPA axis integrates physical and psychosocial influences in order to allow an organism to adapt effectively to its environment, use resources, and optimize survival[36].

FIGURE 3: SYMPTOMS OF LOW TESTOSTERONE



A history of erectile dysfunction, decreased libido, and fatigue may be seen in patients with low testosterone. However, one must realize that these symptoms—as well as others reported by men with low testosterone, such as depression, difficulty concentrating, irritability, and insomnia—are nonspecific and may be related to other medical conditions.[37]

Likewise, physical findings such as muscle weakness, reduced body hair, and altered fat distribution (abdominal obesity) are seen in men with low testosterone, but also in those with a number of other medical conditions.

Additional features suggest specific disorders, eg, anosmia in Kallmann syndrome; eunuchoid body habitus, gynecomastia, and small testes in Klinefelter syndrome[38].

Men with low testosterone may have low bone mineral density or anemia, or both.

Careful examination of the breasts for gynecomastia and the testes for size, consistency, and masses (testicular tumors) helps in formulating a differential diagnosis and in appropriately directing subsequent laboratory evaluation and diagnostic imaging[39]

TYPE 2 DIABETES MELLITUS:

Diabetes mellitus type 2 (also known as type 2 diabetes) is a long term metabolic disorder that is characterized by high blood sugar, insulin resistance, and relative lack of insulin.

Type 2 diabetes primarily occurs as a result of obesity and not enough exercise.[40]Some people are more genetically at risk than others.[41]Type 2 diabetes makes up about 90% of cases of diabetes, with the other 10% due primarily to diabetes mellitus type 1and gestational diabetes.[42] In diabetes mellitus type 1 there is an absolute lack of insulin due to breakdown of islet cells in the pancreas.[43]

Diagnosis of diabetes is by blood tests such as fasting plasma glucose, oral glucose tolerance test, or HbA1C.[44]

Rates of type 2 diabetes have increased markedly since 1960 in parallel with obesity.[44] As of 2013 there were approximately 368 million people diagnosed with the disease compared to around 30 million in 1985.[45] Typically it begins in middle or older age,[45] although rates of type 2 diabetes are increasing in young people.[46,47]Type 2 diabetes is associated with a ten-year-shorter life expectancy.[48]Diabetes was one of

the first diseases described.[48] The importance of insulin in the disease was determined in the 1920s.

Pathophysiology of type 2 diabetes:

Type 2 diabetes is due to insufficient insulin production from beta cells in the setting of insulin resistance.[49] Insulin resistance, which is the inability of cells to respond adequately to normal levels of insulin, occurs primarily within the muscles, liver, and fat tissue.[50]

In the liver, insulin normally suppresses glucose release. However, in the setting of insulin resistance, the liver inappropriately releases glucose into the blood.[51] The proportion of insulin resistance versus beta cell dysfunction differs among individuals, with some having primarily insulin resistance and only a minor defect in insulin secretion and others with slight insulin resistance and primarily a lack of insulin secretion.[51]

Other potentially important mechanisms associated with type 2 diabetes and insulin resistance include:

- increased breakdown of lipids within fat cells,
- resistance to and lack of incretin,
- high glucagon levels in the blood,
- increased retention of salt and water by the kidneys,
- inappropriate regulation of metabolism by the central nervous system.[52]

However, not all people with insulin resistance develop diabetes, since an impairment of insulin secretion by pancreatic beta cells is also required.[52]

DIAGNOSIS OF TYPE 2 DIABETES:

The World Health Organization definition of diabetes (both type 1 and type 2) is for a single raised glucose reading with symptoms, otherwise raised values on two occasions, of either:[53]

- fasting plasma glucose ≥ 7.0 mmol/l (126 mg/dl)

  or

- with a glucose tolerance test, two hours after the oral dose a plasma glucose ≥ 11.1 mmol/l (200 mg/dl)

A random blood sugar of greater than 11.1 mmol/l (200 mg/dL) in association with typical symptoms[54] or a glycated haemoglobin (HbA_{1c}) of ≥ 48 mmol/mol (≥ 6.5 DCCT %) is another method of diagnosing diabetes

Condition	2 hour glucose	Fasting glucose	HbA_{1c}	
Unit	mmol/l(mg/dl)	mmol/l(mg/dl)	mmol/mol	DCCT %
Normal	<7.8 (<140)	<6.1 (<110)	<42	<6.0
Impaired fasting glycaemia	<7.8 (<140)	≥6.1(≥110) & <7.0(<126)	42-46	6.0–6.4
Impaired glucose tolerance	≥7.8 (≥140)	<7.0 (<126)	42-46	6.0–6.4
Diabetes mellitus	≥11.1 (≥200)	≥7.0 (≥126)	≥48	≥6.5

TABLE: WHO DIABETES DIAGNOSIS CRITERIA[55,56]

DOES THE ADIPOSE TISSUE SUPPRESS THE SYNTHESIS OF TESTOSTERONE?

While men with diabetes and/or the metabolic syndrome often have lower-than-normal testosterone levels, the question has arisen whether adipose tissue itself might depress synthesis of testosterone. In recent years, it has been demonstrated that the fat cell functions as an endocrine cell, producing and secreting molecules with regulatory potential, the so-called cytokines/ adipokines of which leptin is a prominent member[57].

Leptin may be a factor in the association between adiposity and decreased testosterone levels. In men, there seems to exist a correlation between body mass index and fat mass on the one hand and leptin levels on the other. Leptin receptors are present on the Leydig cell and inhibit the testosterone generated by administration of human chorionic gonadotropin. This may be a model for a less effective stimulation of testosterone production by luteinizing hormone when circulating leptin levels are high, as is the case of obesity[58].

This finding was supported by studies that found a negative correlation between adiposity, insulin and leptin on the one hand and testosterone levels on the other. More studies have found that insulin is an important determinant of leptin levels[59]. Feeding and overfeeding increase insulin levels which leads to an increase in leptin, and vice-versa.

Hyperinsulinemia, as encountered in insulin resistance, might impair testosterone secretion by the Leydig cell, maybe directly since there are insulin receptors on the Leydig cell[60].

It has also been found in obese men that there is an attenuated pulse amplitude of luteinizing hormone (LH) while the LH pulse frequency is unaffected, thus producing a less strong stimulation of testicular testosterone production.

In morbidly obese men, altered glycosylation of LH, with selective increase in the release of less acidic (biologically inactive) LH isoforms, with a decreased ratio of the biological to immunological ratio of LH, may represent an additional mechanism modulating the hypogonadal state prevailing in morbid obesity[61]. This may explain why the nature of the hypogonadism associated with diabetes mellitus type 2 appears to be hypogonadotropic.

Correlation studies cannot unravel the cause and effect relationships between the correlates whether low testosterone/low SHBG induces visceral fat deposition or whether a large visceral fat depot leads to low testosterone levels. In men with the metabolic syndrome, increases and decreases in body weight are associated with higher/lower insulin levels, with lower/higher SHBG levels and with lower/higher plasma testosterone[62]. Obviously, weight loss will reduce the harmful effects of the metabolic syndrome.

TESTOSTERONE AND INFLAMMATORY STATE :

Pro-inflammatory cytokines such as TNFα, IL-1 and IL-6 are involved in atherogenesis. By contrast, IL-10 and adiponectin are atheroprotective.

Testosterone exerts an immunosuppressive effect on the immune system. Inflammation, infection and trauma reduce testosterone levels as a result of the suppressive action of inflammatory cytokines on the hypothalamic-pituitary-testis axis. Testosterone also directly inhibits cytokine production from lymphocytes.

Administration of testosterone to hypogonadal men with coronary heart disease reduced serum TNFα and IL-1β, but not IL6 levels and raised levels of IL-10. In testosterone deficient men suffering from diabetes type 2[63], baseline testosterone levels inversely correlate with IL-6 and CRP levels. This is also the case in non-diabetic men.

In the study of diabetic men, testosterone replacement had no effect on TNFα, IL-6 or CRP levels. Testosterone did reduce serum leptin and adiponectin levels. This effect may potentially be mediated via reduction in adipose tissue. Similar effects of testosterone replacement in these men have been shown in non-diabetic hypogonadal men.

In a study of hypogonadal men of whom the majority had coronary heart disease testosterone therapy suppressed serum TNFα and IL-1β and increased the anti-atherogenic cytokine interleukin-10 [64]

Adiponectin is atheroprotective and the drop in the level of this cytokine is opposite to the effects of testosterone on other components of the atherogenic cytokine profile[64].

TESTOSTERONE AND ANEMIA :

The role of androgens on erythropoiesis is known since 1941[65] when, before the availability of recombinant human EPO, they represented the main pharmacologic agents in the treatment of anemia of chronic and end-stage renal disease, as well as aplastic anemia[66]

In vitro and animal studies have suggested a role for androgens in increasing erythroid mass, EPO synthesis, and iron bioavailability. In bone marrow cultures, androgens stimulate hematopoiesis[67] also improving erythroid and myeloid colony formation in semisolid cultures[68].

However, myelo stimulating effects of androgens seem to target more mature erythroid progenitors rather than hematopoietic stem cells or immature progenitor cells [69]. In human cell cultures, Testosterone exerts a direct role on bone marrow erythroblasts and red cell precursor survival [68,69,70], by binding with a nuclear androgen receptor (AR) [71] . Interestingly, the pretreatment of bone marrow cells with cyproterone and flutamide, selective competitive blockers of nuclear AR [72], completely inhibits the beneficial effects of Testosterone on erythroid burst-forming units and colony-forming units. In mice, Testosterone administration increases Hb levels and hematocrit as well as granulocyte and platelet numbers, with a greater effect in the oldest experimental study group [73].

Most of the erythropoietic activities of Testosterone seem to be mediated by the stimulation of EPO secretion [74] and by the modulation of erythroid progenitor cells sensitivity to EPO, overall resulting in an augment of the red cells production.

In vitro studies have documented stimulatory properties for androgens on red cell synthesis, blocked by injection of anti-EPO antibody [75]. Furthermore, erythroid progenitor cells from mice treated with T exhibited an increased proliferative response to EPO [75,76]. However, a modest increase in EPO concentrations has been also observed in patients who underwent total androgen blockade therapy (short-term and long-term), after the decline in serum Hb levels, independently of serum Testosterone concentration [76].

There is evidence that Testosterone increases EPO levels by modulating the hypoxia or hypoxic sensing, known to stimulate EPO secretion [76]. Therefore, many effects induced by Testosterone (including increased Hb and hematocrit, increased red cell 2,3-bisphosphoglycerate, and increased muscle capillarity) increase the net oxygen delivery to the tissue.

Alternatively, Testosterone could influence EPO secretion via direct effects at renal peritubular fibroblasts level, establishing a new EPO/Hb "set point." A similar phenomenon is observed in post-transplant erythrocytosis, renal dysfunction, and some populations who live at high altitude. In contrast, other data describe a direct action of Testosterone in hypoxia-induced EPO synthesis.

These studies show no effect of Testosterone on EPO transcription in Hep3B cells, an EPO-secreting cell line highly sensitive to hypoxic stimuli[77].

However, mechanisms other than EPO have been proposed to explain the role of Testosterone deficiency in the decline of both Hb and hematocrit levels. Animal [76,77] and human [78] studies reported that Testosterone may influence iron bioavailability and utilization.

Guo and colleagues [78] recently argued that the increased transferrin levels and reduced serum ferritin concentration could explain the increased Hb concentration observed after Testosterone administration. However, Testosterone replacement therapy with Testosterone propionate seems more effective in stimulating erythropoiesis when chronic administration is established.

It is very well known that serum transferrin concentration reflects the body iron status and erythroid transferrin uptake as well as total erythroid activity. An

alternative hypothesis suggests a role for the suppression of hepcidin, the iron-regulating hormone factor, in the Testosterone -related erythrocytosis. Testosterone might reduce hepcidin levels by decreasing inflammatory cytokines, especially interleukin-6 (IL-6) . Elevated levels of IL-6 are known to negatively influence erythropoiesis, also increasing the liver production of hepcidin through the HAMP gene. It is very well documented that Testosterone exerts anti-inflammatory properties by inhibiting the proinflammatory nuclear transcription factor kappa B (NFκB) and the expression of the inflammatory mediator IL-6[79].

Bone marrow cell cultures treated with serum from patients affected by chronic disease exhibited a suppression of erythroid colony-forming units (CFU-E). Interestingly, this effect reversed after the administration of antibodies against TNFα and IFNγ. In erythroid cells treated with Testosterone, an increase in both iron export from the spleen and iron availability for Hb synthesis has been observed as consequence of the suppression of hepcidin and the upregulation of ferroportin (the major cellular iron exporter).

Consistently, studies in mice with genetic alterations in iron regulatory genes, including inactivating mutations in the gene encoding hepcidin (HAMP), have shown a transient polycythemia[80].

METHODOLOGY

This study was conducted in patients presenting to department of internal medicine at KLE Dr. Prabhakar kore Hospital, Belgaum over a period of January 2015 to December 2015.

Study design:

Case control study

Sample size:

75 cases,75 controls.

Duration:

One year, from January 2015 to December 2015

Sampling procedure:

P1= Po(OR/1) + po(OR-1)

80% sensitivity

10% error

Alpha – 0.05, beta – 0.2

P – Sensitivity – as obtained from previous studies

OR – Odds ratio (As per data provided by MRD, KLE hospital Belgaum)

INCLUSION CRITERIA:

All type 2 detected male diabetic patients. (ADA criteria)

ADA Criteria

1. Fasting plasma glucose ≥126 mg/dl (fasting is defined as no calorie intake for at least 8 hrs) or
2. post prandial plasma glucose ≥200 mg/dl (2 hrs after 75 mg of oral glucose) or
3. symptoms of diabetes plus random blood glucose concentration ≥200mg/dl or
4. GlycatedHb% ≥6.5%

EXCLUSION CRITERIA:

Patients with acute renal failure, cirrhosis, glucocorticoid therapy, HIV infection.

ETHICAL CLEARENCE

Prior to the beginning, the study was approved by the Institutional Ethics Committee, Jawaharlal Nehru Medical College Belagavi.

INFORMED CONSENT

The patients who fulfilled the selection criteria were informed about the nature of study and a written informed consent was obtained (annexure-1)

DATA COLLECTION

Patients were interviewed and demographic data, history of present illness, other co-morbid conditions, personal history were obtained. Further these patients

underwent clinical examination followed by systemic examination and laboratory investigations. These findings were noted on a predesigned and pretested proforma (annexure-2).

LABORATORY INVESTIGATIONS:

- Complete blood count with peripheral smear
- Urea, Sr. Creatinine
- Serum testosterone
- Blood sugar levels – FBS, PPBS
- Sr.Iron, TIBC, Sr. Ferritin
- HbA1c
- Stool for occult blood
- FSH, LH (if necessary).

Serum total testosterone level measured by ELISA

Principle of the test- principle of of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabelled antigen (present in standards , controls and patients samples) and an enzyme –labelled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, enzyme substrate is added. The enzymatic reaction is terminated by by addition of the stopping solution. The absorbance is measured on

a microtiter plate reader. The intensity of the colour formed is inversely proportional to the concentration of the testosterone in the sample. A set of standards is used to plot a standard curve from which the amount of testosterone in patient samples and controls can be directly read[81].

PROCEDURE OF TEST

1. working solutionsof the testosterone-HRP conjugate and wash buffer have prepared.
2. Hundred microwell strips were removed.
3. 50 µlof each calibrator, control and specimen sample pipetted into correspondingly labelled wells in duplicate.
4. 100 µlof the conjugate working solution pipetted into each well.
5. Incubated on a plate shaker (approximately 200 rpm) for 1 hour at room temperature.
6. Each well have washed 3 times with 300 µl of diluted wash buffer l and taped the plate firmly against absorbent paper to ensure that it was dry
7. 150 µlof TMB substrate pipetted into each well at timed intervals.
8. Incubated the plate on a plate shaker at room temperature for 15-20 minutes.
9. 50 µlof stopping solution pipetted into each well at the same timed intervals as in step 7[82].

10 Reading of the plate on a micro well plate reader at 450 nm within 20 minutes after addition of the stoppingsolution has been done.

STATISTICAL METHODS:

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. The following assumptions on data is made, **Assumptions: 1.**Dependent variables should be normally distributed, 2.Samples drawn from the population should be random, Cases of the samples should be independent.

Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups (Inter group analysis) on metric parameters.

Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups.

Pearson's correlation test:

When Pearson's r is close to 1:

This means that there is a strong relationship between your two variables. This means that changes in one variable are strongly correlated with changes in the second variable[83].

When Pearson's r is close to 0:

This means that there is a weak relationship between your two variables. This means that changes in one variable are not correlated with changes in the second variable[83].

When Pearson's r is positive (+):

This means that as one variable increases in value, the second variable also increase in value. Similarly, as one variable decreases in value, the second variable also decreases in value. This is called a positive correlation.

When Pearson's r is negative (-):

This means that as one variable increases in value, the second variable decreases in value. This is called a negative correlation.

If the Sig (2-Tailed) value is greater than 0.05

You can conclude that there is no statistically significant correlation between your two variables. That means, increases or decreases in one variable do not significantly relate to increases or decreases in your second variable.

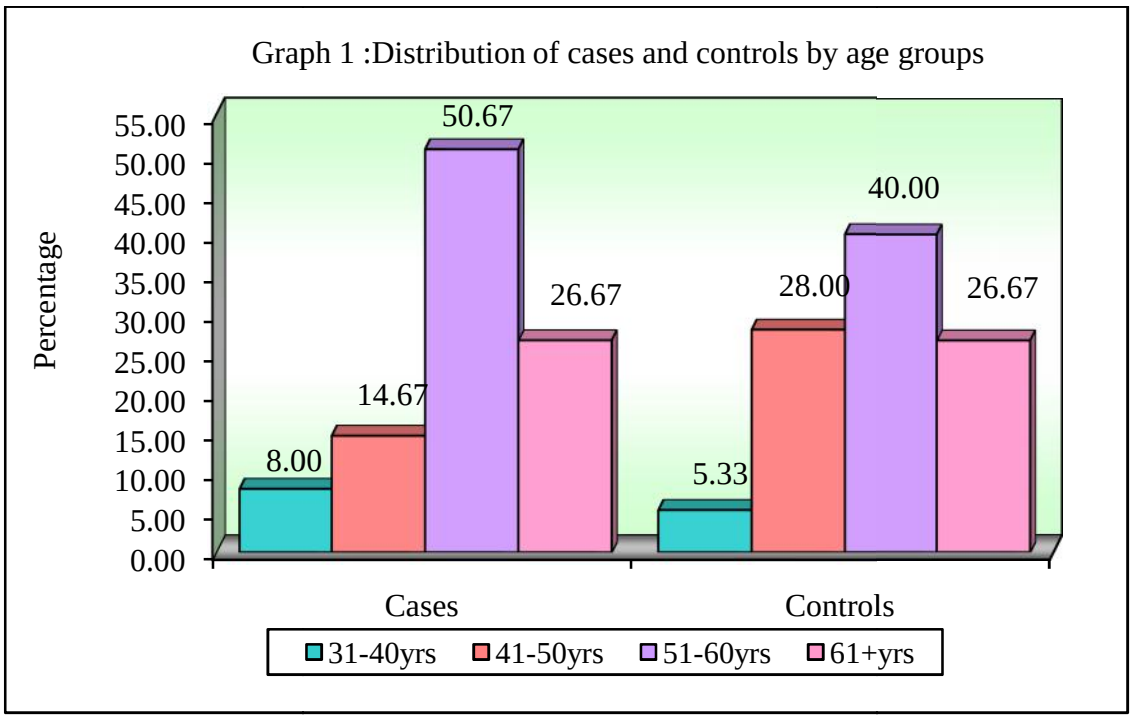
If the Sig (2-Tailed) value is less than or equal to 0 .05

You can conclude that there is a statistically significant correlations between your two variables. That means, increases or decreases in one variable do significantly relate to increases or decreases in your second variable[84].

RESULTS

TABLE 1: DISTRIBUTION OF CASES AND CONTROLS BY AGE GROUPS

Age groups	Cases	%	Controls	%	Total	%
31-40yrs	6	8.00	4	5.33	10	6.67
41-50yrs	11	14.67	21	28.00	32	21.33
51-60yrs	38	50.67	30	40.00	68	45.33
61+yrs	20	26.67	20	26.67	40	26.67
Chi-square=4.4662 P = 0.2153						
Total	75	100.00	75	100.00	150	100.00
Mean age	55.75		55.95		55.85	
SD age	8.98		10.13		9.54	

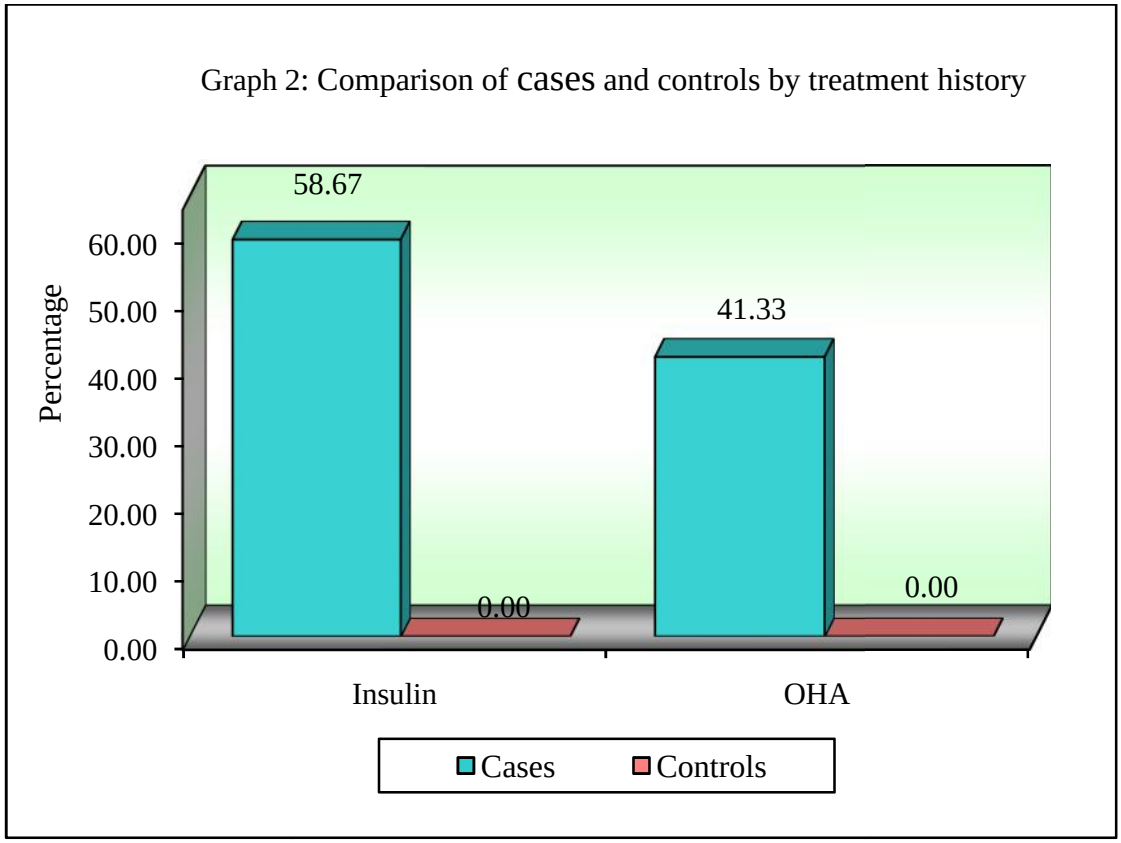


The distribution of age group into cases and controls shows that mean age group in both are 55.75 years, most of subjects are in 51 to 60 years range.

TABLE 2: COMPARISON OF CASES AND CONTROLS BY TREATMENT

Treatment history	Cases	%	Controls	%	Total	%
Insulin	44	58.67	0	0.00	44	29.33
OHA	31	41.33	0	0.00	31	20.67
None	0	0.00	75	100.00	75	50.00
Total	75	100.00	75	100.00	150	100.00
Chi-square=150.0002 P = 0.0001*						

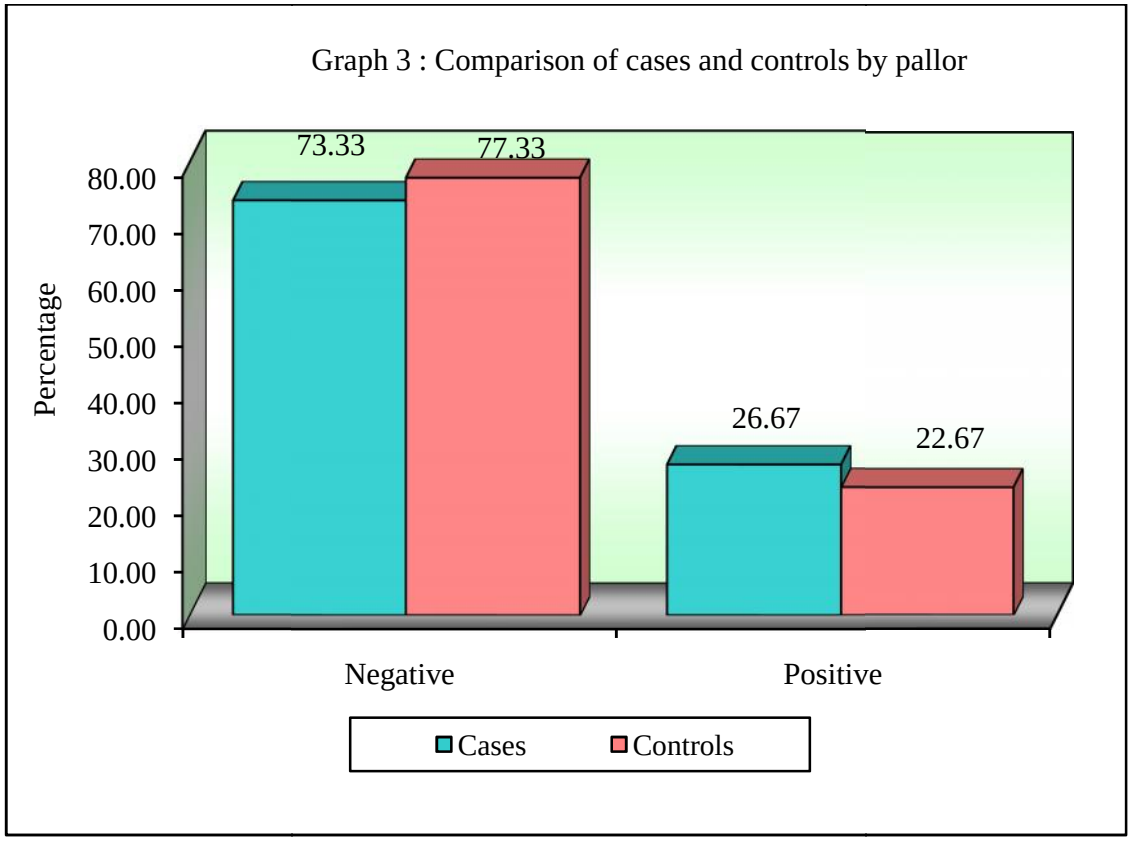
*p<0.05



In this graph shows diabetic patients on insulin and oral hypoglycemic agents, 58.67% were on insulin and 41.33% were on oral hypoglycemic agents.

TABLE 3: COMPARISON OF CASES AND CONTROLS BY PALLOR

Pallor	Cases	%	Controls	%	Total	%
Negative	55	73.33	58	77.33	113	75.33
Positive	20	26.67	17	22.67	37	24.67
Total	75	100.00	75	100.00	150	100.00
Chi-square=0.3235 P = 0.5702						

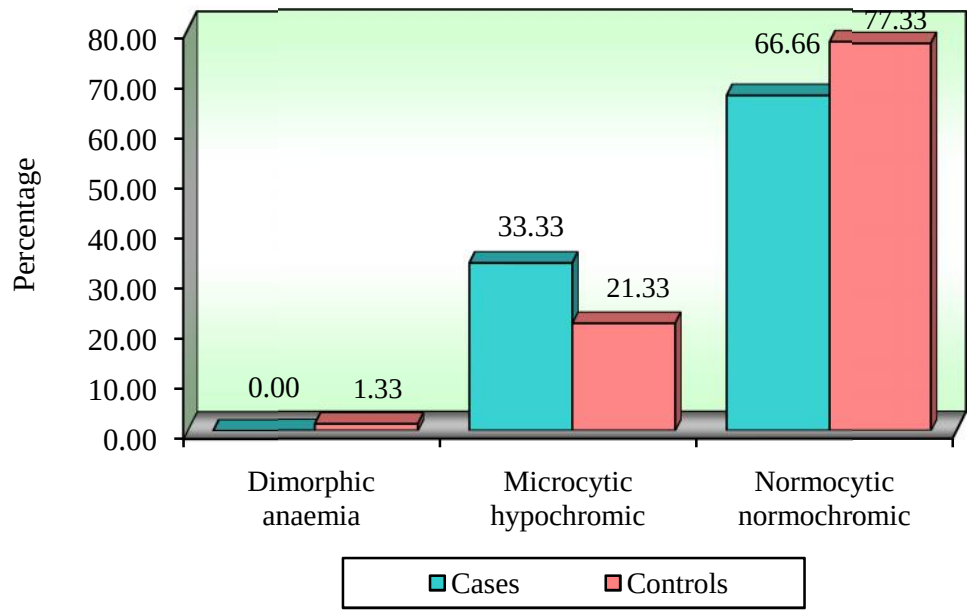


This graph shows distribution of clinical feature pallor in cases and controls, 26.67% cases had pallor, whereas, 22.67% controls had clinical pallor.

TABLE 4: COMPARISON OF CASES AND CONTROLS BY TYPE OF PERIPHERAL SMEAR

Type of Peripheral smear	Cases	%	Controls	%	Total	%
Dimorphic anaemia	0	0.00	1	1.33	1	0.67
Microcytic hypochromic	25	33.33	16	21.33	41	27.33
Normocytic normochromic	50	66.66	58	77.33	108	72.00
Total	75	100.00	75	100.00	150	100.00
Chi-square=3.5683 P = 0.1682						

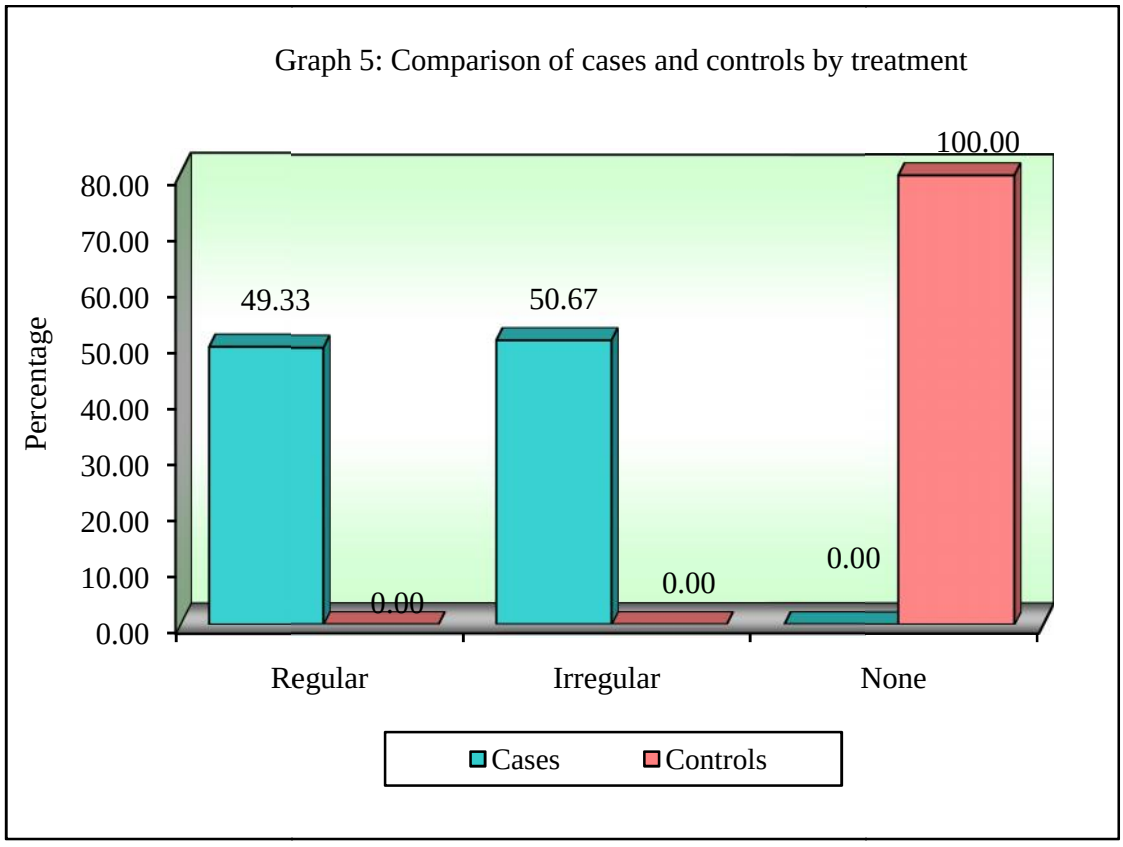
Graph 4 : Comparison of cases and controls by Peripheral smear



In this table and graph shows distribution of subjects based on peripheral smear, 33.33% had microcytic hypochromic anemia, 66.66% had normocytic normochromic anemia in cases. 21.33% and 77.33% had microcytic and normochromic anemia in controls.

TABLE 5: COMPARISON OF REGULAR VERSUS IRREGULAR TREATMENT IN DIABETIC CASES

Treatment	Cases	%	Controls	%	Total	%
Regular	37	49.33	0	0.00	37	24.67
Irregular	38	50.67	0	0.00	38	25.33
None	0	0.00	75	100.00	75	50.00
Total	75	100.00	75	100.00	150	100.00



This graph shows regularity of treatment in diabetic cases, 50.67% were on irregular treatment and 49.33% were on regular treatment.

TABLE 6: CLINICAL FEATURES OF LOW TESTOSTERONE AND LOW HEMATOCRIT IN DIABETICS.

Clinical features	Total (n=75)		P value	
	Low testosterone (n=54)	%		Low hematocrit (n=21)
Decrease in libido	14	25.92%	0.124	6
fatigue	8	14.81%	0.225	2

This table shows clinical features of hypogonadism and its correlation with low testosterone, decreased libido was seen in 14cases, i.e 25.92% of diabetic patients.And fatigue was found in 8 cases, i.e. 14.81% of cases. Total of 22 cases had clinical hypogonadism.

TABLE 7: COMPARISON OF CASES AND CONTROLS WITH DIFFERENT CLINICAL PARAMETERS.

Variables	Cases		Controls		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
BMI (body mass index)	24.15	2.44	23.72	2.11	1.1680	0.2447
Hemoglobin (g/dl)	11.94	1.78	12.20	1.75	-0.8984	0.3704
Hematocrit (%)	41.81	4.26	42.92	3.07	-1.8352	0.0685
Testosterone (ng/dl)	187.42	145.01	406.91	135.55	-9.5760	**0.0001***
FBS(mg/dl)	187.15	66.06	84.27	13.59	13.2114	**0.0001***
PPBS (mg/dl)	268.91	86.91	129.63	14.96	13.6778	**0.0001***
SR. iron(microg/dl)	82.80	38.09	93.77	26.70	-2.0416	**0.0430***
TIBC (microg/dl)	330.19	94.02	299.96	86.48	2.0492	**0.0422***
SR.Ferritin(ng/ml)	113.35	87.02	102.63	52.74	0.9125	0.3630
HBA1C	9.64	2.28	4.48	0.77	18.5643	**0.0001***
Creatinine (mg/dl)	1.29	0.75	1.00	0.28	3.1037	**0.0023***

*$p<0.05$

Table showing comparison between cases and controls in which testosterone mean levels was found to be 187.42ng/dl and 406.91ng/dl in cases and controls respectively. With significant p value of 0.0001. Mean blood sugars were more in cases than controls, with significant p value.

TABLE 8: ASSOCIATION BETWEEN CASES AND CONTROLS WITH DIFFERENT CLINICAL PARAMETERS

Factors	Cases	%	Controls	%	Total	Chi-square	p-value
BMI (body mass index)							
Normal	43	57.33	50	66.67	93	1.3865	0.2390
Obese	32	42.67	25	33.33	57		
HEMOGLOBIN (G/DL)							
Normal	28	37.33	29	38.67	57	0.0283	0.8664
Anemia	47	62.67	46	61.33	93		
HEMATOCRIT							
Normal	54	72.00	68	90.67	122	8.6066	**0.0034***
Reduced	21	28.00	7	9.33	28		
TESTOSTERONE (NG/DL)							
Normal	21	28.00	73	97.33	94	77.0517	**0.0001***
Low testosterone	54	72.00	2	2.67	56		
SR. IRON(microg/dl)							
Normal	44	58.67	71	94.67	115	25.1926	**0.0001***
Reduced	31	41.33	4	5.33	35		
TIBC(microg/dl)							
Normal	52	69.33	62	82.67	114	3.6550	0.0559
Not normal	23	30.67	13	17.33	36		
SR.FERRITIN(ng/ml)							
Normal	71	94.67	75	100.00	146	2.3116	0.1284
Not normal	4	5.33	0	0.00	4		
HBA1C							
Normal	0	0.00	68	90.67	68	120.7586	**0.0001***
Diabetic	75	100.00	7	9.33	82		

*$p<0.05$

This table shows comparison of various parameters, serum testosterone were low in cases that is diabetic subjects with 54 cases, i.e. 72% of total population and controls had 2.67% of hypogonadism, this shows there is significant reduction in testosterone levels in cases than non diabetic controls, with p value of 0.0001.

TABLE 9: HEMATOCRIT LEVELS OF PATIENTS WITH DIABETES IN CASE GROUP.

HEMATOCRIT (%)	TOTAL NO. OF PATIENTS
<40	20 (26.6%)
40 TO 50	54 (72%)
>50	01 (1.33%)
TOTAL	75 (100%)

TABLE 10: TESTOSTERONE LEVEL OF PATIENTS WITH DIABETES IN CASE GROUP.

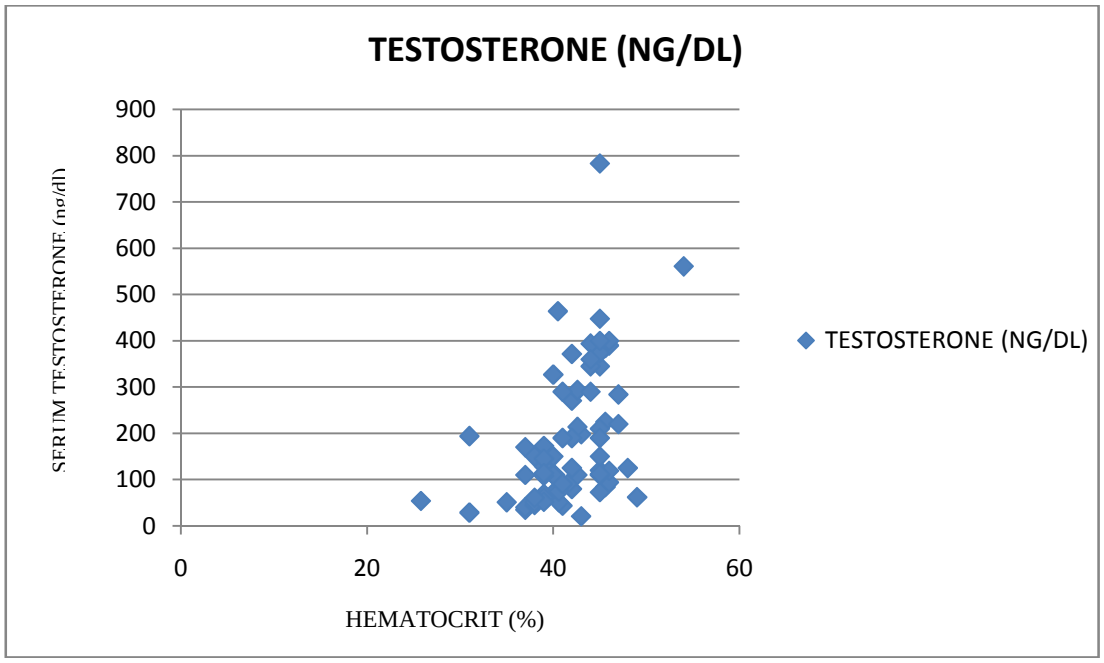
SERUM TESTOSTERONE(ng/dl)	TOTAL NO. OF PATIENTS
241 TO 827	20 (26.66%)
<241	55 (73.33%)
TOTAL	75

Table showing total distribution of diabetic cases 72% of patients had hematocrit of 40 to 50%, and 55 cases had low levels of serum testosterone.

TABLE11: CORRELATION BETWEEN TESTOSTERONE AND HEMATOCRIT IN DIABETIC CASES

		HEMATOCRIT	TESTOSTERONE (NG/DL)
HEMATOCRIT	Pearson Correlation (R)	1	0.454**
	Sig. (2-tailed) (P)		0.000042
	N (total)	75	75
TESTOSTERONE (NG/DL)	Pearson Correlation	**0.454****	1
	Sig. (2-tailed)	**0.000042**	
	N	75	75
**. Correlation is significant at the 0.01 level (2-tailed).			

GRAPH 6: CORRELATION BETWEEN TESTOSTERONE AND HEMATOCRITIN DIABETIC CASES



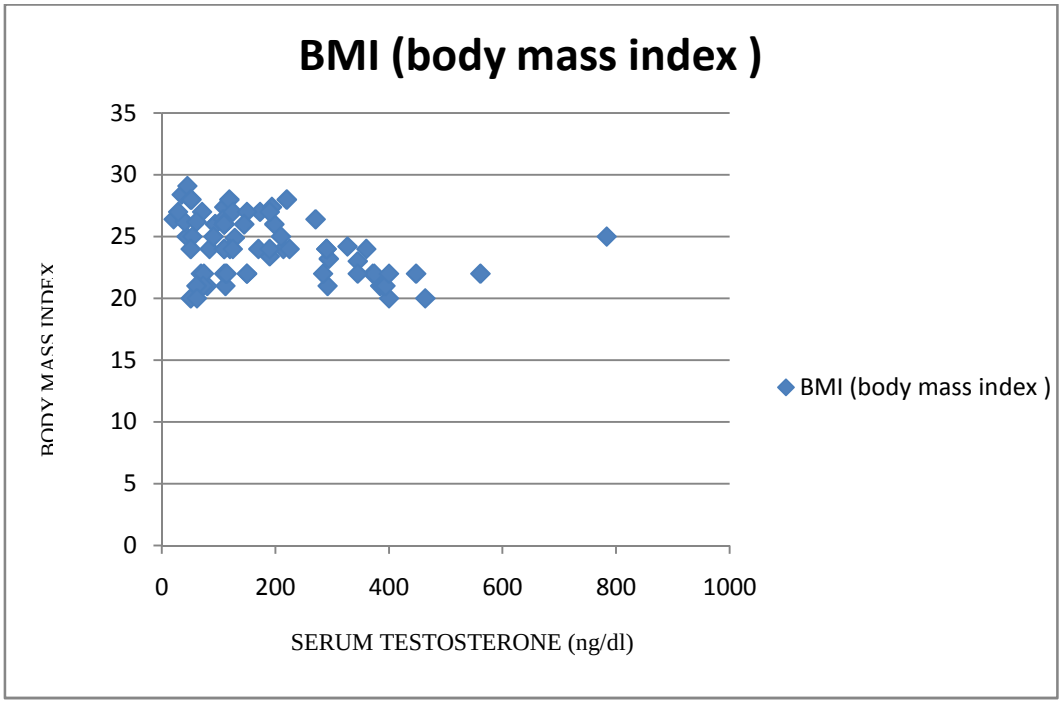
P = 0.000042

In this correlation between hematocrit and testosterone in diabetic patients, there is signicant correlation between two variables shown by pearson table, having r = 0.454, and P = 0.000042, which is significant, as, serum testosterone increases there is reduction in hematocrit. Scatter graph showing distribution of patients in various hematocrit levels having linear correlation.

TABLE12 : CORRELATION BETWEEN TESTOSTERONE AND BMI IN DIABETIC CASES

		TESTOSTERONE (NG/DL)	BMI (body mass index)
TESTOSTERONE (NG/DL)	Pearson Correlation, r	1	-.367**
	Sig. (2-tailed) p		.001
	N	75	75
BMI (body mass index)	Pearson Correlation	**-.367****	1
	Sig. (2-tailed)	**.001**	
	N	**75**	75
**. Correlation is significant at the 0.01 level (2-tailed).			

GRAPH 7:CORRELATION BETWEEN TESTOSTERONE AND BMI IN DIABETIC CASES



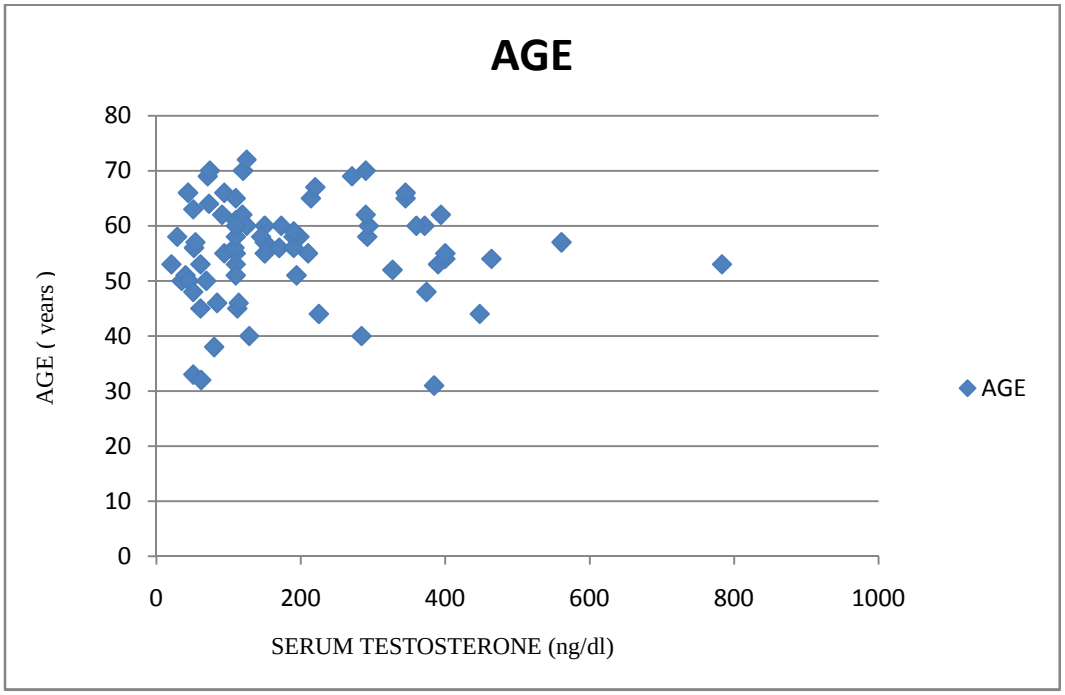
P = 0.0012

Pearson corelation table shows r value of -0.367, which means as BMI increases there is significant reduction in testosterone levels, with p value of 0.0012, statistically significant.

TABLE13: CORELATIONS BETWEEN SERUM TESTOSTERONE WITH AGE IN DIABETIC CASES

		TESTOSTERONE (NG/DL)	AGE
TESTOSTERONE (NG/DL)	Pearson Correlation(r)	1	.006
	Sig. (2-tailed)(p)		.960
	N	75	75
AGE	Pearson Correlation(r)	**.006**	1
	Sig. (2-tailed)(p)	**.960**	
	N(total)	**75**	75

GRAPH 8:CORELATIONS BETWEEN SERUM TESTOSTERONE WITH AGE IN DIABETIC CASES



P = 0.960

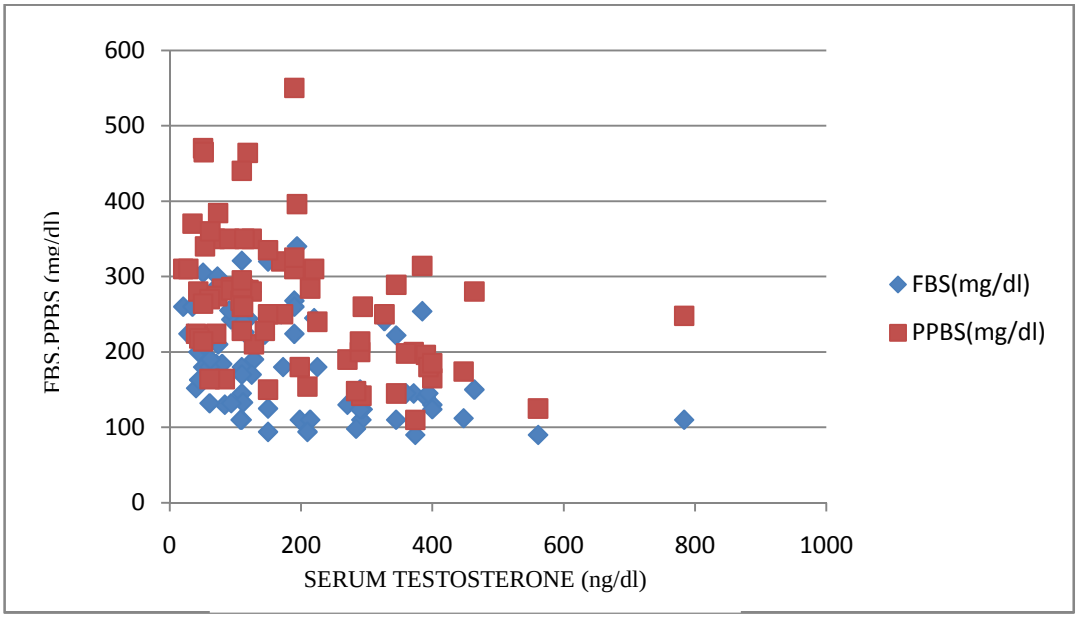
This table shows correlation between age and serum testosterone levels, with r value of 0.006 and p value of 0.960, which are statistically not significant.

TABLE14: CORELATIONS BETWEEN FBS AND PPBS WITH SERUM TESTOSTERONE

		TESTOSTERONE (NG/DL)	FBS(mg/dl)	PPBS(mg/dl)
TESTOSTERONE (NG/DL)	Pearson Correlation	1	-.431**	-.423**
	Sig. (2-tailed)		.000	.000
	N(total)	75	75	75
FBS(mg/dl)	Pearson Correlation(r)	**-.431****	1	.765**
	Sig. (2-tailed)(p)	**.00011**		.000
	N(total)	**75**	75	75
PPBS(mg/dl)	Pearson Correlation(r)	**-.423****	.765**	1
	Sig. (2-tailed)(p)	**.00011**	.000	
	N(total)	**75**	75	75

**. Correlation is significant at the 0.01 level (2-tailed).

GRAPH 9:CORELATIONS BETWEEN FBS AND PPBS WITH SERUM TESTOSTERONE



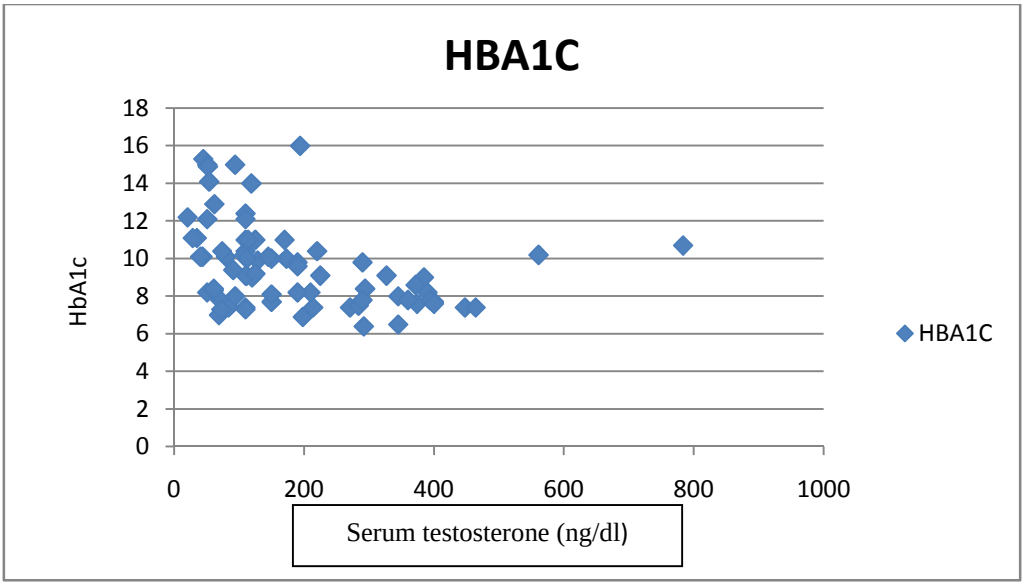
P = 0.00011

This is correlation between blood sugars FBS and PPBS with testosterone levels, the r =-0.423 and p =0.0001, this suggests that, as FBS and PPBS increases there is reduction in serum testosterone levels. There is significant correlation between two parameters.

TABLE15: CORRELATION BETWEEN HBA1C WITH SERUM TESTOSTERONE

		TESTOSTERONE (NG/DL)	HBA1C
TESTOSTERONE (NG/DL)	Pearson Correlation	1	-.365**
	Sig. (2-tailed)		.001
	N	75	75
HBA1C	Pearson Correlation(r)	**-.365****	1
	Sig. (2-tailed)(p)	**.001**	
	N(total)	**75**	75
**. Correlation is significant at the 0.01 level (2-tailed).			

GRAPH 10: CORRELATION BETWEEN HBA1C WITH TESTOSTERONE.



P = 0.0012

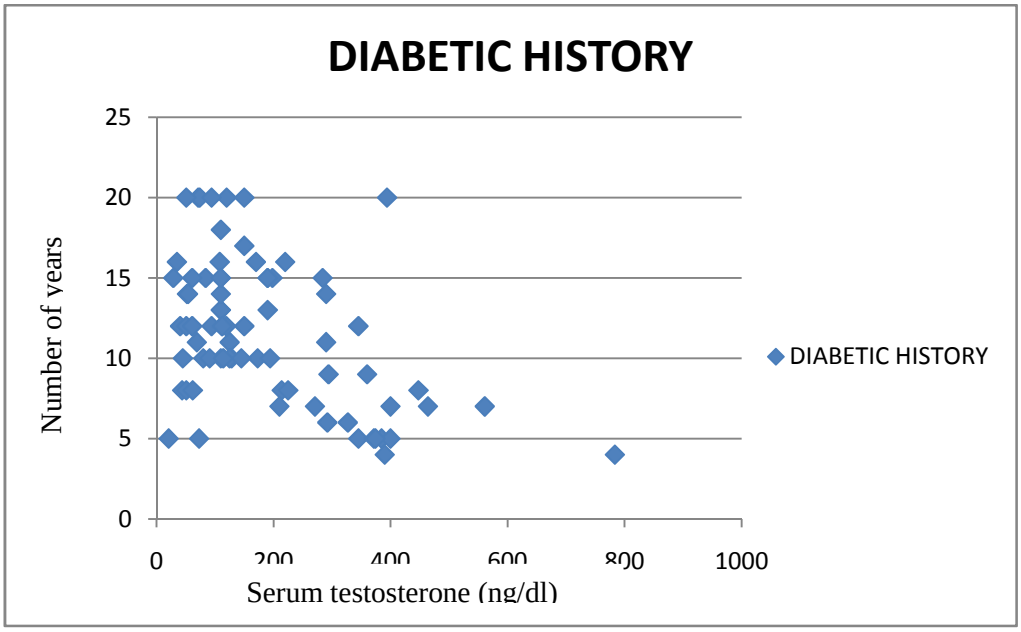
On correlating HbA1c with testosterone levels, using pearson correlation, r= -0.365, p = 0.0012, which is statistically significant. As, HbA1c increases there is significant reduction in testosterone levels.

TABLE 16: SENSITIVITY OF TESTOSTERONE AS A MARKER FOR LOW HEMATOCRIT VALUE

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
TESTOSTERONE (NG/DL) * HEMATOCRIT	75	**92.6%**	6	7.4%	81	100.0%

This table shows sensitivity of serum testosterone with hematocrit which 92.6% sensitive.

GRAPH 11: CORELATION BETWEEN DURATION OF DIABETES WITH SERUM TESTOSTERONE LEVELS.



P = 0.000018

There is significant corelation between serum testosterone and duration of diabetes with p = 0.000018, as duration of diabetes increases, there is reduction in testosterone levels.

DISCUSSION

In our study 75 patients with diabetes and 75 controls. (nondiabetics) who visited Prabhakar Kore hospital, Belgaum and compared there testosterone levels and hematocrit levels, and also correlated them with other parameters.

Prevalence of testosterone,

The present study, we found that low testosterone level was seen in 72% of diabetic patients, as compared with controls of 2.67%, using a cut off of 241 ng/dl. Having significant p value of 0.0001.

Several studies have clearly established that low serum testosterone is common in men with type 2 diabetes, along with inappropriately low levels of hematocrit values. Several cross sectional studies and systemic analyses have reported that type 2 diabetes is associated with low serum testosterone. Tomar R et al[85], Kapoor D et al[86], DhindsaS et al[87], Basu A et al[88], Ayman A[89], Grossman M et al[90], have prevalence of low serum testosterone is 48%,17%, 33%, 40%, 36%, 43% respectively.

The decrease in testosterone is slow and constant over all decades and starts early in life, probably after the third or fourth decade. The exact cause of the age-related reduction in testosterone levels is not known.

Anemia and low testosterone

In our study the haematocrit values among diabetics 28% had low haematocrit value, using a cut off of less than 40%. 20 patients had haematocrit value of <40%, 54 patients had value 40 to 50%, and 01 patient had value more than 50%. We also correlated serum testosterone with haematocrit values in diabetic subjects which showed p value of 0.000042. which is highly significant. As the level of serum testosterone decreases there is high proportion of reduction in haematocrit values.

In a study by grossmann et al[90], .Individuals with total testosterone level < 10 nmol/l (43% of the cohort) were more likely to have anaemia . Similarly, anaemia was twice as common in individuals with a calculated free testosterone of < 0.23 nmol/l (adjusted odds ratio 2.0, 95% CI 1.2-3.1).

We studied haemoglobin levels, 47 subjects had low haemoglobin in diabetics compared to 46 subjects in nondiabetics. i.e. 62.67% and 61.33% respectively.
With a p value of 0.8664. In a study by grossman et al, demonstrate that low testosterone levels are independently associated with anaemia in men with type 2 diabetes.

In our study we did iron studies, mean sr. iron was 82.80, and iron stores were reduced in 31 cases with low testosterone and diabetes, accounting for 41.33%. And mean sr.ferritin was 113.35, and 5.33% had low ferritin. In a study by grossman et al, shows that testosterone levels significantly contribute to Hb variability and anaemia, independent and additive to the to the actions of chronic kidney disease, iron storage and systemic inflammation.

In our study normocytic normochromic anemia was found in 66.66% of diabetics,i.e. 50 cases, and microcytic hypochromic anemia was found in 25 cases i.e. 33.33% of diabetic population. When compared with nondiabetic controls, 58 patients

i.e. 77.33% had normocytic normochromic anemia, whereas 16 subjects, i.e. 21.33% had microcytic hypochromic anemia in controls. This data suggests that there is significant degree of microcytic hypochromic anemia in diabetic cases. Serum iron studies between two groups revealed there was significant difference between iron stores in two study population with mean iron of 82.80 in diabetics and 93.77 in nondiabetic controls. 41.33% had low iron levels in cases compared to 5.33% in controls.

Interestingly, in our study, anaemia was significantly more common in the setting of reduced iron availability when testosterone levels were also low.

BMI and low testosterone

The present study shows 42.67% were obese diabetics and 33.33% in non obese diabetic patients. On correlating BMI with seum testosterone levels there was p value of 0.0012, which was significant. As, BMI increases the testosterone levels were less. Not only obese but lean diabetics also have low tesotosterone, significant with other studies. Corona G et al reported that the presence of low testosterone was not entirely dependent upon obesity because 25% of non obese patients (31% of lean and 21% of overweight) also had hypogonadism.Sabooraftab SA et al[91], and dhindsas et al[87] have shown a significant association between BMI and low serum testosterone level.

Age and low testosterone

dhindsas et al[87] have shown a significant association between age and low testosterone levels. In our study, when we compared between diabetics versus normal individuals, most study population were in the age group of 51 to 60 years in the both

study group. Accounting for 50.67% in cases and 40 % in age related controls. Mean age among both groups was 55.95.when we correlated age and serum testosterone there was p value of 0.960, not that significant compared to previous studies, probably because our study included more of middle aged between 51 to 60 years compared to studies by Kapoor et al and dhinsda et al, which included more of elderly population with mean age of 70 years and above.

Blood sugars and low testosterone

Many studies have not compared blood glucose levels with testosterone in diabetics, but our study depicts it, mean FBS in diabetics was 187 mg/dl, and in non diabetics was 84 mg/dl, and mean PPBS of diabetics and controls were 268 mg/dl and 129 mg/dl repectively.

On correlating blood sugar levels with testosterone, p value was found to be 0.0001, which is very significant. Our study depicts that as blood sugar level increases (FBS) there is considerable reduction in testosterone levels. . In our study on correlating HbA1c and serum testosterone levels in diabetics there was significant correlation, with p value of 0.0012 and mean HbA1c was 9.64. As, poor the glycemic control, there is significant decrease in testosterone levels.

DinsadaS et al[87] , GrossmnM et al[90] and Ayman A et al[89], showed there was no significance between HbA1c and testosterone levels which is in contrast with those by Kapooret al[86] and Fukui et al[95], in which testosterone concentrations correlated positively with HbA1c concentrations.

Symptoms of hypogonadism and low testosterone :

In our study, decreased libido was seen in 14 cases of diabetes, i.e 25.92% of low testosterone patients, p value was not significant in our study, and fatigue was seen in 8 cases, i.e. 14.81% of low testosterone diabetics, with non significant p value. In a study done by grossman et al, they did not take symptomatology into account, and they concluded that, generalised symptomatology in individuals with anaemia is almost impossible to distinguish from those of hypogonadism. In other study by dhinsda et al, there was correlation with p value of 0.001 with hypogonadism and low testosterone levels. Not only clinical but lab parameters are also necessary for testosterone deficiency, and to intervene early.

Sensitivity of hematocrit and low testosterone as a marker was 92.6%, which was found in our study.

In this study, we demonstrate that low testosterone levels are independently associated with anemia in men with type 2 diabetes, these data confirm the previous association between androgens and hemopoiesis in men with type 2 diabetes[91]. In addition we show that testosterone levels significantly contribute to Hb variability and anemia, independent and additive to the actions of iron storage, systemic inflammation.

CONCLUSION

In our study, showed that a significant number with type 2 diabetes have testosterone deficiency.

1. The prevelance of low testosterone in diabetes was 72%
2. There is significant correlation between testosterone and hematocrit in diabetics, as testosterone decreases there is significant decrease in hematocrit.
3. Low levels of testosterone were associated with high BMI, poor glycemic control and long standing diabetes.

Low Testosterone can be one of the predictive value for determining insulin resistance and metabolic syndrome, can be taken as indirect marker for hypogonadism in type 2 diabetes after excluding the cause.

Given the overlapping symptomatology with anemia, this suggests those presenting with anemia should also be screened for testosterone deficiency.

SUMMARY

The proposed study was carried out in 150 patients as case control study, cases being diabetics and controls being normal age matched non diabetic patients attending the prabhakarkore hospital, Belgaum.

All the patients were subjected to detailed history taking, clinical examination and were subjected to blood investigations with informed consent.

The results were tabulated and serum testosterone was estimated and it is correlated with hematocrit in type 2 diabetes male patients and statistically evaluated using appropriate methods.

We found,

- Low testosterone in 72% of population.
- Normocytic normochromic anemia was found in 66.66% of cases.
- There was significant correlation with p=0.000042 among testosterone and hematocrit values in cases.
- There was significant correlation between testosterone and hematocrit levels and also outcome was significant with BMI, blood sugar levels, duaration of diabetes, HbA1c levels.
- 50.67% o population were on irregular treatment.
- Clinically decrease in libido was seen in 14 cases, fatigue was seen in 8 cases.
- Sensitivity of hematocrit and low testosterone as a marker was 92.6%.

BIBLIOGRAPHY

1. Haffner SM, Miettinen H, Karhapä P et al. Leptin concentrations, sex hormones, and cortisol in nondiabetic men. J ClinEndocrinolMetab1997; 82: 1807-9.
2. Shores MM, Matsumoto AM, Sloan KL, Kivlahan DR. Low serum testosterone and mortality in male veterans. *Arch Intern Med* 2006; 166: 1660-5.
3. Selvin E, Feinleib M, Zhang L et al. Androgens and diabetes in men.

   Diabetes Care2007; 30: 234-8.

4. R. D. Stanworth, T. H. Jones, "Testosterone in obesity, metabolic syndrome and type 2 diabetes," Frontiers of Hormone Research, vol. 37, pp. 74–90, 2009.
5. R. D. Stanworth, D. Kapoor, K. S. Channer, T. H. Jones, "Statin therapy is associated with lower total but not bioavailable or free testosterone in men with type 2 diabetes," Diabetes Care, vol. 32, no. 4, pp. 541–546, 2009.
6. Laaksonen DE, Niskanen L, Punnonen K, *et al.* Sex hormones, inflammation and the metabolic syndrome: a population based study. Eur J Endocrinol 2003;149:601–8.
7. Kupelian V, Page ST, Araujo AB, *et al.* Low sex hormone binding globulin, total testosterone, and symptomatic androgen deficiency are associated with development of the metabolic syndrome in non obese men. J ClinEndocrinolMetab 2006; 91:843–50.
8. Hak AE, Witteman JCM, De Jong FH, *et al.* Low levels of endogenous androgens increase the risk of atherosclerosis in elderly men: the Rotterdam Study. J ClinEndocrinolMetab 2002;87:3632–9.

9. Laughlin GA, Barrett-Connor E, Bergstrom J. Low serum testosterone and mortality in older men. J ClinEndocrinolMetab 2008;93:68–75.

10. Rosner, W., Auchus, R.J., Azziz, R. et al. (2007) Position statement: utility, limitations, and pitfalls in measuring testosterone: an Endocrine Society position statement. Journal of Clinical Endocrinology and Metabolism, 92, 405-413.

11. Vermeulen, A., Verdonck, L. & Kaufman, J.M. (1999) A critical evaluation of simple methods for the estimation of free testosterone in serum. Journal of Clinical Endocrinology and Metabolism, 84, 3666-3672.

12. Vermeulen, A. (2005) Hormonal cut-offs of partial androgen deficiency: a survey of androgen assays. Journal of Endocrinological Investigation, 28, 28-31.

13. Beutler, E. &Waalen, J. (2006) The definition of anemia: what is the lower limit of normal of the blood hemoglobin concentration? Blood, 107, 1747-1750.

14. Bhasin S, Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, Swerdloff RS, Montori VM. Testosterone therapy in adult men with androgen deficiency syndromes: an endocrine society clinical practice guideline. J ClinEndocrinolMetab 91:1995–2010, 2006.

15. Calof O, Singh AB, Lee ML, Urban RJ, Kenny AM,Tenover JL, Bhasin S. Adverse events associated with testosterone supplementation of older men: a metaanalysisof randomized, placebo-controlled trials. J Gerontol A Med Sci 60:1451–1457, 2005.

16. Bhasin S, Woodhouse L, Casaburi R, Singh AB, Bhasin D, Berman N, Chen X, Yarasheski KE, Magliano L, Dzekov C, Dzekov J, Bross R, Phillips J, Sinha-

Hikim I, Shen R, Storer TW Testosterone dose-response relationships in healthy young men. Am J PhysiolEndocrinolMetab 281:E1172–E1181, 2001.

17. Muram D, et al. Comparability of single measurements of serum testosterone to the 24-hour cavg in patients using testosterone 2% solution. J Sex Med 2014 11(11): p. 2826-9.

18. Muram D, et al. Skin reactions in a phase 3 study of a testosterone topical solution applied to the axilla in hypogonadal men. Curr Med Res Opin 2012 28(5): p. 761-6.

19. Wang C, et al. Efficacy and safety of the 2% formulation of testosterone topical solution applied to the axillae in androgen-deficient men. ClinEndocrinol (Oxf) 2011 75(6): p. 836-43.

20. Salehian B, et al. Pharmacokinetics, bioefficacy, and safety of sublingual testosterone cyclodextrin in hypogonadal men: comparison to testosterone enanthate--a clinical research center study. J ClinEndocrinolMetab 1995 80(12): p. 3567-75.

21. Hall SA, et al. Correlates of low testosterone and symptomatic androgen deficiency in a population-based sample. J ClinEndocrinolMetab 2008 93(10): p. 3870-7.

22. Nieschlag E, et al. Testosterone: action, deficiency, substitution.Cambridge University Press. 2004 ISBN 9780521833806

23. Parker KL, et al. Genes essential for early events in gonadal development. Cell Mol Life Sci 1999 55(6-7): p. 831-8.

24. Brinkmann AO. Molecular mechanisms of androgen action--a historical perspective. Methods Mol Biol 2011 776: p. 3-24.

25. Bentvelsen FM, et al. The androgen receptor of the urogenital tract of the fetal rat is regulated by androgen. Mol Cell Endocrinol 1994 105(1): p. 21-6.

26. Singh J, et al. Induction of spermatogenesis by androgens in gonadotropin-deficient (hpg) mice. Endocrinology 1995 136(12): p. 5311-21.

27. Sun YT, et al. The effects of exogenously administered testosterone on spermatogenesis in intact and hypophysectomized rats. Endocrinology 1989 125(2): p. 1000-10.

28. McLachlan RI, et al. Hormonal regulation of spermatogenesis in primates and man: insights for development of the male hormonal contraceptive. J Androl 2002 23(2): p. 149-62.

29. Weinbauer GF, et al. Gonadotrophin-releasing hormone analogue-induced manipulation of testicular function in the monkey. Hum Reprod 1993 8 Suppl 2: p. 45-50.

30. deRonde W, et al. Aromatase inhibitors in men: effects and therapeutic options. ReprodBiolEndocrinol 2011 9: p. 93.

31. Brinkmann AO. Molecular basis of androgen insensitivity. Mol Cell Endocrinol 2001 179(1-2): p. 105-9

32. Zitzmann M. Mechanisms of disease: pharmacogenetics of testosterone therapy in hypogonadal men. Nat ClinPractUrol 2007 4(3): p. 161-6.

33. Rajender S, et al. Phenotypic heterogeneity of mutations in androgen receptor gene. Asian J Androl 2007 9(2): p. 147-791

34. Canale D, et al. Androgen receptor polymorphism (CAG repeats) and androgenicity. ClinEndocrinol (Oxf) 2005 63(3): p. 356-61.

35. Kaufman JM, et al. The decline of androgen levels in elderly men and its clinical and therapeutic implications. Endocr Rev 2005 26(6): p. 833-76.

36. Malenka RC, Nestler EJ, Hyman SE (2009). "Chapter 10: Neural and Neuroendocrine Control of the Internal Milieu". In Sydor A, Brown RY. Molecular Neuropharmacology: A Foundation for Clinical Neuroscience (2nd ed.). New York: McGraw-Hill Medical. pp. 246, 248–259.

37. Kapoor D, Aldred H, Clark S, et al.Clinical and biochemical assessment of hypogonadism in men with type 2 diabetes.Correlations with bioavailable testosterone and visceral adiposity. Diabetes Care 2007;30:911–17.

38. Haffner SM, Shaten J, Stern MP, et al. Low levels of sex hormone-binding globulin and testosterone predict the development of non-insulin-dependent diabetes mellitus in men. MRFIT Research Group. Multiple Risk Factor Intervention Trial. Am J Epidemiol 1996;143:889–97.

39. Selvin E, Feinleib M, Zhang L, et al. Androgens and diabetes in men. Results from the Third National Health and Nutrition Survey (NHANES III). Diabetes Care 2007;30:234–8.

40. "Diabetes Blue Circle Symbol". International Diabetes Federation. 17 March 2006.

41. "Causes of Diabetes". National Institute of Diabetes and Digestive and Kidney Diseases. June 2014. Retrieved 10 February 2016.

42. Tfayli, H; Arslanian, S (March 2009). "Pathophysiology of type 2 diabetes mellitus in youth: the evolving chameleon.". Arquivosbrasileiros de endocrinologia e metabologia.**53** (2): 165–74. doi:10.1590/s0004-27302009000200008. PMC 2846552.PMID 19466209.

43. Imperatore, Giuseppina; Boyle, James P.; Thompson, Theodore J.; Case, Doug; Dabelea, Dana; Hamman, Richard F.; Lawrence, Jean M.; Liese, Angela D.; Liu,

Lenna L. (2012-12-01). "Projections of Type 1 and Type 2 Diabetes Burden in the U.S. Population Aged <20 Years Through 2050". Diabetes Care. 35 (12): 2515–2520.

44. Williams textbook of endocrinology. (12th ed.). Philadelphia: Elsevier/Saunders. pp. 1371–1435. ISBN 978-1-4377-0324-5.

45. Ripoll, Brian C. Leutholtz, Ignacio (2011-04-25). Exercise and disease management(2nd ed.). Boca Raton: CRC Press. p. 25. ISBN 978-1-4398-2759-8.

46. Zaccardi, F; Webb, DR; Yates, T; Davies, MJ (February 2016). "Pathophysiology of type 1 and type 2 diabetes mellitus: a 90-year perspective.". Postgraduate Medical Journal. **92**(1084): 63–9. doi:10.1136/postgradmedj-2015-133281. PMID 26621825.

47. Vijan, S (2010-03-02). "Type 2 diabetes". Annals of Internal Medicine.**152** (5): ITC31–15; quiz ITC316. doi:10.7326/0003-4819-152-5-201003020-01003.PMID 20194231.

48. Ripsin CM, Kang H, Urban RJ (January 2009). "Management of blood glucose in type 2 diabetes mellitus". Am Fam Physician. **79** (1): 29–36. PMID 19145963.

49. Pasquier, F (October 2010). "Diabetes and cognitive impairment: how to evaluate the cognitive status?". Diabetes & metabolism. 36 Suppl 3: S100–5. doi:10.1016/S1262-3636(10)70475-4. PMID 21211730.

50. Risérus U, Willett WC, Hu FB (January 2009). "Dietary fats and prevention of type 2 diabetes". Progress in Lipid Research. **48** (1): 44–51.doi:10.1016/j.plipres.2008.10.002. PMC 2654180. PMID 19032965.

51. Touma, C; Pannain, S (August 2011). "Does lack of sleep cause diabetes?".Cleveland Clinic journal of medicine. **78** (8): 549–58. doi:10.3949/ccjm.78a.10165.PMID 21807927.

52. Christian, P; Stewart, CP (March 2010). "Maternal micronutrient deficiency, fetal development, and the risk of chronic disease". The Journal of Nutrition. 140 (3): 437 45.doi:10.3945/jn.109.116327. PMID 20071652.

53. Abdullah, A; Peeters, A; de Courten, M; Stoelwinder, J (September 2010). "The magnitude of association between overweight and obesity and the risk of diabetes: a meta-analysis of prospective cohort studies.". Diabetes research and clinical practice. 89 (3): 309 19. doi:10.1016/j.diabres.2010.04.012. PMID 20493574

54. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation (PDF). Geneva: World Health Organization. 2006. p. 21.ISBN 978-92-4-1594936.

55. Vijan, S (March 2010). "Type 2 diabetes". Annals of Internal Medicine. 152 (5): ITC31-15.doi:10.7326/0003-4819-152-5-201003020-01003. PMID 20194231.

56. World Health Organization. "Definition, diagnosis and classification of diabetes mellitus and its complications: Report of a WHO Consultation. Part 1. Diagnosis and classification of diabetes mellitus". Retrieved 2007-05-29.

57. Pitteloud N, Dwyer AA, DeCruz S, et al. Inhibition of luteinizing hormone secretion by testosterone in men requires aromatization for its pituitary but not its hypo-thalamic effects: evidence from the tandem study of normal and gonadotropin-releasing hormone-deficient men. J ClinEndocrinolMetab 2008;93:784–791.

58. Hayes FJ, DeCruz S, Seminara SB, Boepple PA, Crowley WF. Differential regulation of gonadotropin secretion by testosterone in the human male: absence of a negative feedback effect of testosterone on follicle-stimulating hormone secretion. J ClinEndocrinolMetab 2001; 86:53–58.

59. Hayes FJ, Pitteloud N, DeCruz S, Crowley WF, Boepple PA. Importance of inhibin B in the regulation of FSH secretion in the human male. J ClinEndocrinolMetab 2001; 86:5541–5546.

60. Pitteloud N, Dwyer AA, DeCruz S, et al. The relative role of gonadal sex steroids and gonadotropin-releasing hormone pulse frequency in the regulation of follicle-stimulating hormone secretion in men. J ClinEndocrinolMetab 2008; 93:2686–2692.

61. Cooke RR, McIntosh JE, McIntosh RP. Circadian variation in serum free and non-SHBG-bound testosterone in normal men: measurements, and simulation using a mass action model. ClinEndocrinol (Oxf) 1993; 39:163–171.

62. Diver MJ, Imtiaz KE, Ahmad AM, Vora JP, Fraser WD. Diurnal rhythms of serum total, free and bioavailable testosterone and of SHBG in middle-aged men compared with those in young men. ClinEndocrinol (Oxf) 2003; 58:710–717.

63. Clair P, Claustrat B, Jordan D, Dechaud H, Sassolas G. Daily variations of plasma sex hormone-binding globulin binding capacity, testosterone and luteinizing hormone concentrations in healthy rested adult males. Horm Res 1985; 21:220–223.

64. Woolf PD, Hamill RW, McDonald JV, Lee LA, Kelly M. Transient hypogonadotropic hypogonadism caused by critical illness. J ClinEndocrinolMetab 1985;60:444–450.

65. J. C. Schooley, “Inhibition of erythropoietic stimulation by testosterone in polycythemic mice receiving anti-erythropoietin,” Proceedings of the Society for Experimental Biology and Medicine, vol. 122, no. 2, pp. 402–403, 1966. View at Publisher · View at Google Scholar · View at Scopus

66. M. Golfam, R. Samant, L. Eapen, and S. Malone, "Effects of radiation and total androgen blockade on serum hemoglobin, testosterone, and erythropoietin in patients with localized prostate cancer," Current Oncology, vol. 19, no. 4, pp. e258–e263, 2012. View at Publisher · View at Google Scholar · View at Scopus

67. V. H. Haase, "Regulation of erythropoiesis by hypoxia-inducible factors," Blood Reviews, vol. 27, no. 1, pp. 41–53, 2013. View at Publisher · View at Google Scholar · View at Scopus

68. W. Guo, E. Bachman, M. Li et al., "Testosterone administration inhibits hepcidin transcription and is associated with increased iron incorporation into red blood cells," Aging Cell, vol. 12, no. 2, pp. 280–291, 2013. View at Publisher · View at Google Scholar · View at Scopus

69. X. Pan, N. Suzuki, I. Hirano, S. Yamazaki, N. Minegishi, and M. Yamamoto, "Isolation and characterization of renal erythropoietin-producing cells from genetically produced anemia mice," PLoS ONE, vol. 6, no. 10, Article ID e25839, 2011. View at Publisher · View at Google Scholar · View at Scopus

70. F. Lainé, B. Laviolle, M. Ropert et al., "Early effects of erythropoietin on serum hepcidin and serum iron bioavailability in healthy volunteers," European Journal of Applied Physiology, vol. 112, no. 4, pp. 1391–1397, 2012. View at Publisher · View at Google Scholar · View at Scopus

71. K. L. Blanchard, A. M. Acquaviva, D. L. Galson, and H. F. Bunn, "Hypoxic induction of the human erythropoietin gene: cooperation between the promoter and enhancer, each of which contains steroid receptor response elements," Molecular and Cellular Biology, vol. 12, no. 12, pp. 5373–5385, 1992. View at Publisher · View at Google Scholar · View at Scopus

72. M. Gross and E. Goldwasser, “On the mechanism of erythropoietin-induced differentiation. XIV. The apparent effect of etiocholanolone on initiation of erythropoiesis,” Experimental Hematology, vol. 4, no. 4, pp. 227–233, 1976. View at Google Scholar · View at Scopus

73. W. Guo, E. Bachman, M. Li et al., “Testosterone administration inhibits hepcidin transcription and is associated with increased iron incorporation into red blood cells,” Aging Cell, vol. 12, no. 2, pp. 280–291, 2013. View at Publisher · View at Google Scholar · View at Scopus

74. E. Bachman, R. Feng, T. Travison et al., “Testosterone suppresses hepcidin in men: a potential mechanism for testosterone-induced erythrocytosis,” Journal of Clinical Endocrinology and Metabolism, vol. 95, no. 10, pp. 4743–4747, 2010. View at Publisher · View at Google Scholar · View at Scopus

75. D. P. Delev, D. P. Davcheva, I. D. Kostadinov, and I. I. Kostadinova, “Effect of testosterone propionate on erythropoiesis after experimental orchiectomy,” Folia Medica, vol. 55, no. 2, pp. 51–57, 2013. View at Google Scholar · View at Scopus

76. K. Punnonen, K. Irjala, and A. Rajamäki, “Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency,” Blood, vol. 89, no. 3, pp. 1052–1057, 1997. View at Google Scholar ·View at Scopus

77. E. Nemeth, S. Rivera, V. Gabayan et al., “IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin,” The Journal of Clinical Investigation, vol. 113, no. 9, pp. 1271–1276, 2004. View at Publisher · View at Google Scholar · View at Scopus

78. W. B. Ershler, “Biological interactions of aging and anemia: a focus on cytokines,” Journal of the American Geriatrics Society, vol. 51, no. 3, supplement, pp. S18–S21, 2003. View at Publisher · View at Google Scholar · View at Scopus

79. T. J. Fry, M. Moniuszko, S. Creekmore et al., "IL-7 therapy dramatically alters peripheral T-cell homeostasis in normal and SIV-infected nonhuman primates," Blood, vol. 101, no. 6, pp. 2294–2299, 2003. View at Publisher · View at Google Scholar · View at Scopus

80. A. C. Cooper, A. Mikhail, M. W. Lethbridge, D. M. Kemeny, and I. C. Macdougall, "Increased expression of erythropoiesis inhibiting cytokines (IFN-gamma, TNF-alpha, IL-10, and IL-13) by T cells in patients exhibiting a poor response to erythropoietin therapy," Journal of the American Society of Nephrology, vol. 14, no. 7, pp. 1776–1784, 2003.

81. Argyrous, G. Statistics for Research: With a Guide to SPSS. London: SAGE. ISBN 1-4129-1948-7.

82. Bryman, Alan; Cramer, Duncan (2011). Quantitative Data Analysis with IBM SPSS 17, 18 and 19: A Guide for Social Scientists. New York: Routledge. ISBN 978-0-415-57918-6.

83. Levesque, R. (2007). SPSS Programming and Data Management: A Guide for SPSS and SAS Users (4th ed.). Chicago, Illinois: SPSS Inc. ISBN 1-56827-390-8.

84. SPSS 15.0 Command Syntax Reference. Chicago, Illinois: SPSS Inc. 2006.

85. Tomar R, Dhindsa S, Chaudhuri A, Mohanty P, Garg R, Dandona P. Contrasting testosterone concentrations in type 1 and type 2 DM. Diabetes Care 2006;29:1120-22.

86. Kapoor D, Aldred H, Clark S, Kevin S, Channer T, Jones H. Correlations with bioavaible testosterone and visceral adiposity. Diabetes Care 2007; 30:911

87. Dhindsa S, Prabhakar S, Sethi M, Bandyopadhyay A, Chaudhuri A, Dandona P. Frequent occurrence of hypogonadotropic hypogonadism in type 2 diabetes. J ClinEndocrinolMetab2004; 89(11):5462–8

88. Basu A , Singhania P , Bandyopadhyay R, Biswas K , Santra S , Sundhakar et al. Late onset hypogonadism in type 2 diabetic and non diabetic male, a comparative study. J Indian Med assoc 2012;110:573-75.

89. Ayman A, Hayek A, Yousef S, Khader, Sahar J, Khawaja N et al. prevalence of hypogonadism in type 2DM. J Fam Community Med 2013;20:179-86.

90. Grossmann M, Thomas MC, Panagiotopoulos S, Sharpe K, Macisaac RJ, Clarke S, et al. Low testosterone levels are common and associated with insulin resistance in men with diabetes. J ClinEndocrinolMetab 2008;93:1834–40.

91. The role of obesity and type 2 diabetes mellitus in the development of male obesity-associated secondary hypogonadism.Saboor Aftab SA[1], Kumar S, Barber TMClinEndocrinol (Oxf). 2013 Mar;78(3):330-7. doi: 10.1111/cen.12092.

ANNEXURE I – CONSENT FORM

ESTIMATION OF SERUM TESTOSTERONE LEVEL AND ITS CO-RELATION WITH HEMATOCRIT IN TYPE 2 DIABETES MALE PATIENTS - ONE YEAR HOSPITAL BASED PROSPECTIVE CASE-CONTROL STUDY IN KLES DR PRABHAKAR KORE CHARITABLE HOSPITAL & MRC, BELGAUM

Objective and purpose of the study:

. This research is intended to assess **autonomic dysfunction in cirrhosis of liver** The principal investigator of the study is Dr. _____ TURAMARI under the guidance of Dr. _____

Procedure:

If you agree to be part of the research study you will be asked the relevant history and will be subjected to relevant clinical examination and investigations.

Risk and Benefits:

The only risk and possible discomfort you might get is while taking blood from my arm for the investigations. It may cause swelling, pain, redness, bruising or infection (rarely happens) at the site from where the blood is drawn

Alternatives

Taking part in this study is voluntary. You may choose not to take part in this study, or if you decide to take part you can later change my mind and withdraw from the study. Your decision will not change the present or future health care or other services that you receive. The study doctor or sponsorer may stop your participation

in this study any time. If you choose not to take part in the study you will receive the standard treatment for patients with your condition

Privacy and Confidentiality:

All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study may be published but your identity will be confidential in any publication.

Institution / Sponsor's policy:

Does not apply to this research

VOLUNTARY PARTICIPATION/ WITHDRAWAL:

Your participation in this study is entirely voluntary and you may withdraw from the study at any time.

.

Financial incentives for participation

You will not be paid / offered any gifts /incentives for participating in the study.

Authorization to publish the results

The results of the study would be forwarded to the KLE University, Belgaum as part of requirement towards the completion of MD degree, review and publishing.

If you have any questions about your rights as a participant you may call:

DR. _____

Chairman,
J.N.M.C Ethical Committee for Human Research,
Professor and Head , Department of Pathology , JNMC Belagavi
Phone number: _____
Extn: _____

Dr. _____

Professor & HOD,
Department of Medicine,
JNMC, Belagavi.
Phone No: _____
Extn: _____

Dr. _____

Investigator,
PG in General Medicine,
JNMC, Belagavi.
Phone No.: _____

CONSENT FORM

I voluntarily agree to take part in this study by signing on the line below. I may withdraw at any time. I am not giving up any of my legal rights by signing this form. My signature below indicated that I have read this entire consent form or it has been read to me, and has been explained to me in my vernacular language and had all my questions answered. I will be given a copy of this consent form.

Signature /Left Thumb print of the Participant or legally authorized representative.

Participant's Name/ : …….

Signature/ Left Thumb

Impression of the participant's : …….

Name of the legally

authorized representative/ Guardian : …….

Signature/ Left Thumb

Impression. : …….

Witness's Name : …….

Signature/ Left Thumb

Impression. :…….

Investigators name and Signature : …….

Date and Place : …….

ANNEXURE II – PROFORMA

Sl.NO : NAME :

AGE : SEX : OCCUPATION :
RELIGION :

I.P/O.P NO. :

ADDRESS :

DATE OF ADMISSION:

HISTORY :

PAST HISTORY & FAMILY HISTORY :

MEDICAL HISTORY :

GENERAL PHYSICAL EXAMINATION:

- PALLOR :
- ICTERUS :
- CYANOSIS :
- CLUBBING :
- ODEMA :

VITALS : PR : BP :

SYSTEMIC EXAMINATION :

CVS :

RS :

CNS :

PA :

HEMOGLOBIN	
HEMATOCRIT	
WBC	
PLATELET	

PERIPHERALSMEAR	
UREA	
CREATININE	
SR.TESTOSTERONE	
FBS, PPBS	
SR.IRON	
TIBC	
SR. FERRITIN	
HBA1C	
OCCULTBLOOD	

ANNEXURE III –KEY TO MASTER CHART

M	–	Male
IP/OP	–	Inpatient/ outpatient
IHD	–	Ischemic heart disease
AWMI	–	Anterior wall Myocardial Infarction
CVA	–	Cerebro Vascular Accident
BPPV	–	Benign positional paroxysmal vertigo
-	–	Negative
+	-	Positive
mg/dl	–	Milligrams per deciliter
ng/dl	–	nanogram per deciliter
HTN	-	Hypertension

NORMAL VALUES:

1) HEMOGLOBIN : 13 TO 16 g/dL
2) HAEMATOCRIT : 40 TO 50 %
3) HBA1C : <5.6 NORMAL, 5.7 – 6.4 PRE DIABETIC, >6.5 DIABETIC
4) CREATININE: 0.80 – 1.30 mg/dl
5) GLUCOSE FASTING : <100 mg/dl = normal fasting glucose, 100 – 125 = impaired fasting glucose, >126 = provisional diabetes.
6) GLUCOSE RANDOM : <200 mg/d
7) WBC : 4000 – 10000
8) PLATELET COUNT : 150000 – 450000
9) SR. IRON : 65 – 175 microg/dl
10) TIBC : 250 – 450 microg/dl
11) SR. FERRITIN : 22 – 322 ng/ml
12) SR. TOTAL TESTOSTERONE : 241 – 827 ng/dl