PROSTATE SPECIFIC ANTIGEN (PSA), SEX HORMONES AND CALCIUM LEVEL AMONG ADULT MALES WITH DIABETES IN VARIOUS HEALTH FACILITIES EDO STATE

BY

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CERTIFICATION

This is to certify that the work reported in this project work was carried out by **EJEYE BENJAMIN AGHOGHO** under my supervision in partial fulfillment of the requirement of the award of Bachelor of Medical Laboratory Science (BMLS) degree.

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DEDICATION

I dedicate this seminal work to God Almighty, and to all those suffering from any form of glucose metabolism disorder, together with their family members and care givers.

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My profound gratitude goes to God Almighty, whose I am and to whom I belong,
his grace, tender mercies and insights has been more than sufficient to conduct this research.

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CHAPTER ONE

1.0 BACKGROUD OF STUDY

The discovery of serum prostate specific antigen (PSA) and its extensive use over
the past two decades has dramatically influenced the diagnosis and monitoring of prostate cancer before and after treatment, respectively (Dada *et al,* 2018). Prostate specific antigen (PSA) is a 28 400Da glycoprotein (Milford *et al*, 2001) comprising 237 amino acid residues (Lundwall, *et al* 1991) with five inter-chain disulphide bonds and approximately 8% carbohydrate in the form of a N-linked oligosaccharide side chain. In seminal plasma, PSA can be shown to exist in five isoforms, two biologically active and differing in the degree of glycosylation, and three biologically inactive or `nicked' forms (Zhang *et al,* 1999). This inherent heterogeneity has proved problematic in the various purifications, and some procedures have not yielded a product in which all isoforms are represented. Prostate specific antigen exhibits serine protease activity (EC 3.4.21.77) similar to chymotrypsin. Prostate specific antigen is a member of the human kallikrein family, with which it shares considerable structural and functional homology and a gene location on the long arm of chromosome 19 (19q13.2 - q13.4)
(Dada *et al,* 2018).

Prostate specific antigen is synthesized in the ductal and acinar epithelium of the
prostate gland, whence it is secreted into the seminal plasma at a concentration of 0.5-2.0g/L; secretion has been identified in the paraurethral and perianal glands as well as in apocrine sweat glands and the mammary glands. Synthesis of PSA has also been demonstrated in a number of tumour cell-lines, notably neuroblastoma (Mannello *et al*, 2000). The function of PSA is to liquefy the seminal coagulum by proteolysis, with release of the entrapped spermatozoa, and may have a bioactive role in fertilization (Dada *et al,* 2018).

Testosterone is an androgen that is primarily produced by the Leydig cells of the
testes. Obesity results in reduced serum testosterone levels in men
(Hirosh *et al,* 2016). Recent studies have shown that low serum testosterone levels were associated with endothelial dysfunction, insulin resistance, and cognitive dysfunction, which are independent risk factors for cardiovascular diseases, type 2 diabetes mellitus, and dementia. Therefore, it is important for men to prevent a decline in serum testosterone levels (Kapoor *et al,* 2007).
Testosterone is the primary male sex hormone and is synthesized from cholesterol

through a cascade of enzyme reactions. In males, it acts through androgen receptors and it has both androgenic effects including maturation of sex organs and development of male secondary sex characteristics and anabolic effects which include growth of muscle mass and bone maturation
(Bove and Chitnis, 2014).

Follicle stimulating hormone (FSH) and luteinizing hormone (LH) are members of the glycoprotein hormone (GPH) family. They are secreted by gonadotropes which are found in the anterior pituitary gland of the hypothalamus.

In males, FSH stimulate the sertoli cells to produce androgen-binding proteins (ABP) which keeps testicular testosterone concentrations elevated for spermatogenesis and inhibin which through a feedback mechanism to the anterior pituitary gland, stimulates the cease in production of FSH. In females, FSH stimulates ovarian follicular granulose cells to continue to grow and divide, secrete estradiol and become responsive to LH stimulation.

LH in males stimulates testicular Leydig cells to synthesize and secrete testosterone, while in females; LH stimulates ovarian theca cells to produce the androgen precursor converted to estradiol by granulose cells. LH surge in females triggers ovulation and stimulates progesterone synthesis and release by the corpus luteum during the luteal phase of the menstrual cycle (Pardue *et al.,* 2017).

Prolactin is produced in several tissues such as mammary gland, lymphocytes,

spleen, thymus and adenohypophysis. Prolactin in males act like a growth factor or protective factor for the prostate epithelium by having autocrine or paracrine action, in reproduction, there is negative correlation between sperm and prolactin levels (Maria, 2016). Its hyper secretion in men has been associated with decreased sexual desire, infertility, reduction of testosterone and erectile dysfunction. In females, prolactin stimulates breast milk production during pregnancy (Maria, 2016).

Calcium is an element that plays an important role not only in skeletal mineralization but also in a wide range of biological functions (Peacock, 2010). In recent decades, insulin resistance and secretion have been shown to depend on calcium homeostasis. The secretion of insulin in response to an elevated concentration of plasma glucose is a $Ca2^{+}$-dependent process. Alterations in insulin secretion have also been involved with disorders in blood glucose homeostasis, and increasing cytosolic calcium has been associated with an increase in the expression of GLUT4 transporters in the myocyte, which, in turn, increases the insulin-stimulated glucose transport activity in these cells. Because both defects in insulin secretion and insulin action are related to type 2 diabetes, it is expected that abnormal calcium homeostasis could play an important role in the development of diabetes (American Diabetes Association 2010).

Diabetes mellitus is a metabolic disorder characterized by the presence of

hyperglycemia due to defective insulin secretion, defective insulin action or both. The chronic hyperglycemia of diabetes is associated with relatively specific long-term microvascular complications affecting the eyes, kidneys and nerves, as well as an increased risk for cardiovascular disease (CVD). The diagnostic criteria for diabetes are based on thresholds of glycemia that are associated with microvascular disease, especially retinopathy. "Prediabetes" is a practical and convenient term referring to impaired fasting glucose (IFG), impaired glucose tolerance (IGT) (1) or a glycated hemoglobin (A1C) of 6.0% to 6.4%, each of which places individuals at high risk of developing diabetes and its complications (CDA, 2014).

1.1 JUSTIFICATION OF THE STUDY

Diabetes mellitus (DM) is a serious problem in male health. A positive association exists between clinical markers of benign prostatic hyperplasia (BPH) and DM. Subnormal serum free testosterone is detected in diabetic men. Kasper et al suggested an inverse correlation between DM and the risk of prostate cancer (Kasper et al, 2006).

Recent studies have suggested an association between type 2 diabetes mellitus and lower risk of prostate cancer (Bonovas *et al.,* 2004 and Zhu *et al.,* 2004). It has been hypothesized that men with long-term diabetes have a lower risk of prostate cancer than nondiabetic men, and recently diagnosed men have a higher risk (Rodriguez *et al.,* 2005). In biologic models proposed to explain this

association, researchers note the higher concentrations of insulin and insulin-like growth factor 1 (IGF-1) in early diabetes and the lower testosterone and IGF-1 levels and higher estrogen concentrations in long term diabetes (Djavan *et al.,* 2001 and Betancourt *et al.,* 2003). Whether diabetes influences levels of biomarkers such as prostate-specific antigen (PSA), which is involved in the detection of prostate cancer, is unknown.

Factors influencing serum PSA levels in men include age, benign prostatic

hyperplasia, prostatitis, and body mass index (BMI) (Baillargeon *et al*., 2005). Still, we understand little about PSA, and its relations with comorbid conditions remain unexplored (Game *et al*., 2003). Diabetes and PSA screening are prevalent among men aged 50 years or older, and no doubt many men this age with diabetes undergo PSA testing. In this analysis, we examined whether PSA concentrations varied by diabetes status and duration of diabetes (Thompson *et al.,* 2003)

1.2 AIM OF THE STUDY

The aim of this study is to investigate Prostate Specific Antigen and Testosterone

level in adult diabetic male in Edo State.

1.3 SPECIFIC OBJECTIVES

1. To determine the level of Serum Prostate Specific Antigen in adult diabetic male in Edo State.
2. To determine the level of Sex Hormone in adult diabetic male in Edo State.
3. To determine the level of Calcium in adult diabetic male in Edo State.

CHAPTER TWO

LITERATURE REVIEW

2.1 ANATOMY AND BIOLOGY OF THE PROSTATE

The prostate is a fibromuscular gland surrounding the male urethra. It is located behind the symphysis pubis, inferior to the bladder and anterior to the rectum. The prostate is traversed by the urethra and both ejaculatory ducts. It is divided into anatomical areas and zones. The distal glandular prostate constitutes around 95% of the glandular tissue and is divided into two regions: the peripheral and central zone, comprising nearly 75% and 25% of its volume, respectively. The fibromuscular stroma blends proximally with the detrusor muscle fibers that surround the urethra at the bladder neck. The proximal glandular prostate consists of the transition zone, which accounts for about 5% of the glandular element, and a periurethral zone. It is of clinical importance to distinguish the transition zone from the rest of the prostate (Milford *et al*., 2001).

Whilst it rarely develops a malignant cancer, it is the sole site for the development of benign prostatic hyperplasia (BPH). Histologically, the prostate is composed of non-striated fibromuscular tissue and glandular tissue. Numerous follicles and papillae open into 12-20 excretory ducts. The ductal epithelium is responsible for production and secretion of prostate specific antigen (PSA). The basement membrane creates a barrier preventing escape of PSA into the peripheral circulation. It is the disruption of this barrier by disease which allows an influx of PSA into the systemic circulation

(Milford *et al.*, 2001).

Embryologically, while the prostate utricle is derived from the Mullerian tubes (equivalent of the vagina), the majority of the gland is derived from the urogenital sinus. This is an androgen-dependent process. At puberty the gland doubles in size due to follicular bud branching. The gland grows steadily by epithelial in-folding. After 45-50 years of age, whilst the glandular elements atrophy, the actual gland size increases due to benign hyperplasia of the epithelial and fibromuscular elements. The prostatic fluid forms the first part of the ejaculate, approximately 30% of the overall volume. The prostatic fluid is acidic and rich in acid phosphatases, zinc, magnesium, albumin and calcium. It functions to support the spermatozoa (Milford *et al.*, 2001).

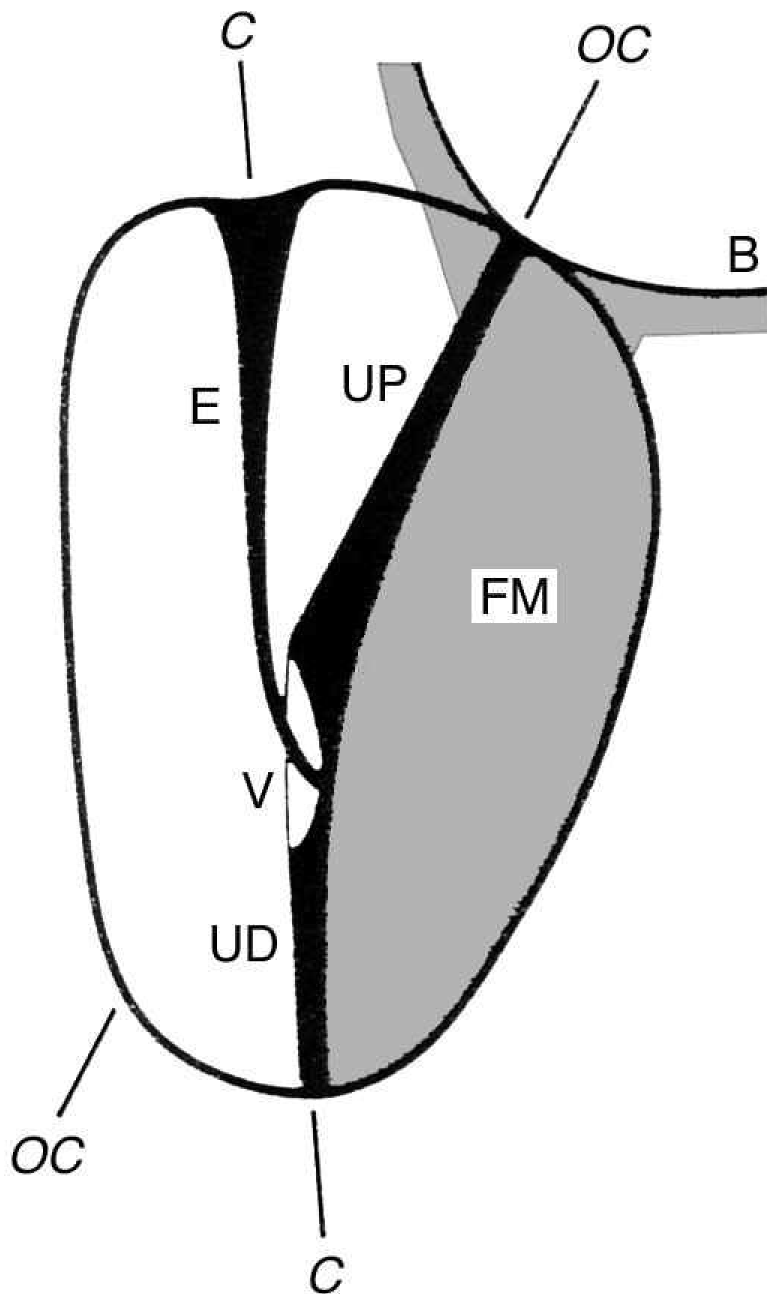

FIGURE 1. *Sagittal diagram of the prostate. The coronal plane (C) is shown in relation to the ejaculatory ducts (E), verumontanum (V) and distal urethral segment (UD). The oblique coronal plane (OC) is shown in relation to the bladder (B) and proximal urethral segment (UP). The shaded area (FM) represents the fibromuscular stroma.*

Source: Milford 2001

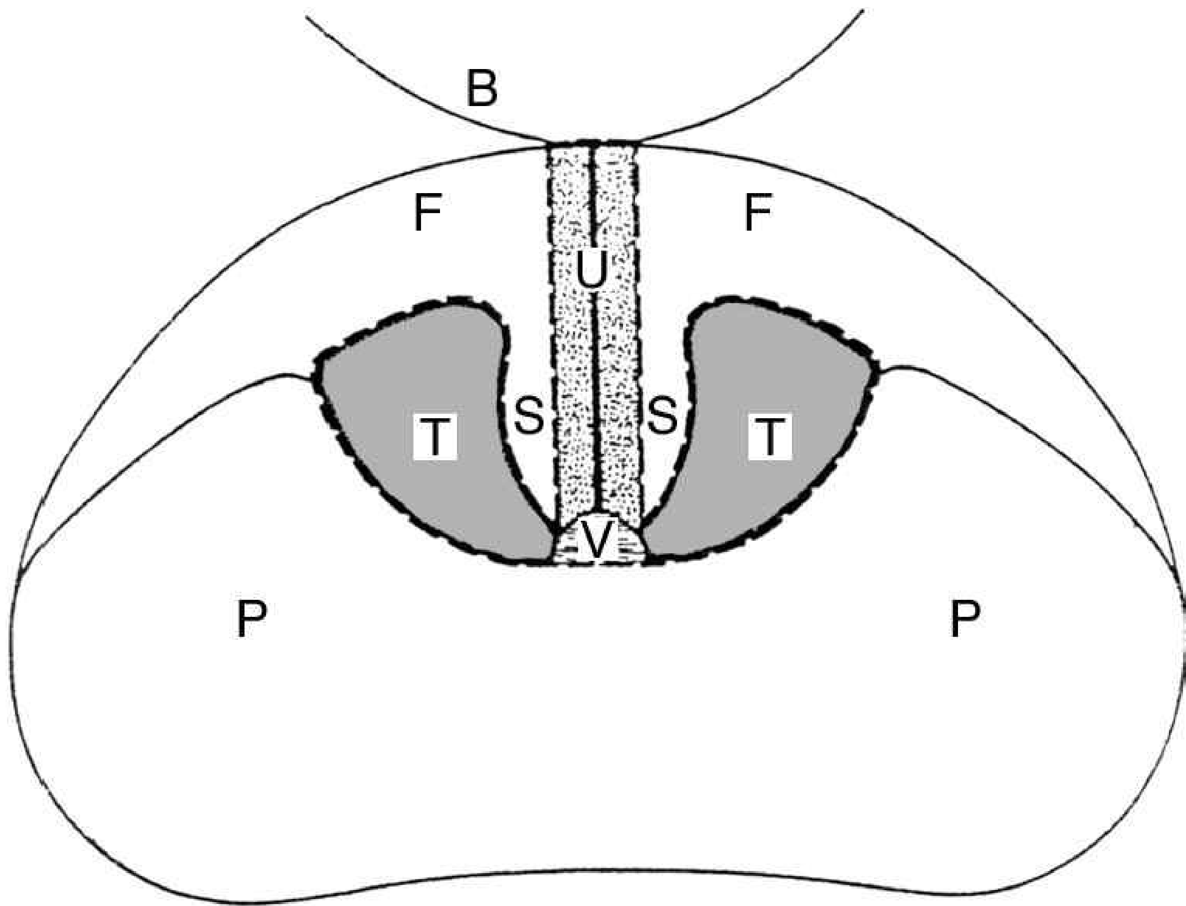

FIGURE 2. *Oblique coronal plane in the prostate. This shows the relationships between the transition zone (T), periurethral region (U), sphincter (S), main glandular prostate (P) and verumontanum (V). The anterior fibromuscular stroma (F), the bladder (B) and the urethra are shown (heavy line). The dashed line surrounds the stippled area representing the region of origin of benign prostatic hyperplasia.*

Source: Milford 2001

2.2 INFLAMMATION OF THE PROSTATE

Inflammation of the prostate can be acute or chronic, bacterial or sterile. Acute bacterial prostatitis is characterized by the abrupt onset of fever, dysuria, haematuria and urinary frequency. Most causative organisms, usually derived from the urinary tract, are sensitive to the quinolone antibiotics e.g. ciprofloxacin (Baillargeon *et al*., 2005).

Whilst modern antibiotics have reduced the incidence of acute prostatitis, its ability to elevate the serum PSA level has led to increased clinical prominence (Hasui *et al*., 2000). Pansadoro *et al.,* 1999*,* found that 71% of men with acute prostatitis had elevations of their serum PSA, mostly in the 4-10 µg/L range. This elevation is usually transient, lasting for about 6 weeks (Tchetgen and Oesterling, 2000). Chronic prostatitis, now increasingly common, runs a more indolent course and is frequently sterile. Serum PSA concentrations are increased in less than 10% of patients (Pansadoro *et al.,* 1999).

Other non-infective causes of prostatic inflammation, including urethral catheterization, acute urinary retention, prostate biopsy and prostatic massage, are also capable of causing an increase in the serum PSA concentration.

Serum PSA concentrations return to normal once the acute inflammation has subsided. Several authors have shown that standard digital rectal

examination produces a small, clinically insignificant rise in the serum PSA

(Walz *et al.,* 1998).

BENIGN PROSTATIC HYPERPLASIA (BPH)

Whilst BPH is one of the most common ailments affecting ageing men, with more than 60% in their sixties having histologically proven disease, only two thirds will develop symptoms. Stromal nodules enlarge and coalesce, producing urethral compression. Subsequently, the bladder undergoes secondary changes resulting in detrusor hypertrophy, fibrosis, diverticula formation and eventually detrusor failure if the disease progresses unchecked (Hasui *et al*., 2000).

Symptoms are best divided into those of outflow obstruction and those of secondary bladder irritation, and are generally referred to as lower urinary tract symptoms (LUTS). Since there is much subjective variation in symptomatology, the objective International Prostate Symptom Score (IPSS) was developed. In uncomplicated cases, investigations are usually limited to digital examination of the prostate, uroflowmetry and measurement of the post-void residual bladder volume (Thompson *et al,* 2003).

Treatment is aimed at symptomatic relief and options vary as to the best approach: watchful waiting, drug therapy and surgery. Pharmacologically, the delta-adrenergic antagonists were known to relieve symptoms and to

have revolutionized the management of BPH (Eri and Tveter, 2000). They act to relax the smooth muscle in the prostate and bladder neck. Other commonly used pharmacological agents include 5-delta reductase inhibitors e.g., finasteride. These act by preventing the conversion of testosterone into its active metabolite dihydrotestosterone (DHT), but appear less efficacious than a blockade

(Vaughan *et al.,* 1999). Surgically, transurethral resection of the prostate (TURP) is the `gold standard' treatment. It has withstood many other competitors (e.g. microwave, laser, and ultrasound) as well as the test of time. Benign prostatic hyperplasia leads to an increase in prostatic size and a subsequent increase in the serum PSA concentration. Stamey *et al.*, 1990, studied the PSA of men with BPH undergoing prostatectomy. Using the Yang Pros-check assay, they attributed

0.3 μg/L of serum PSA to each gram of prostate tissue. Hence, the concept of PSA density was introduced to differentiate a small gland with high PSA (likely to be malignant) and a large gland with a raised PSA appropriate to its size.

ADENOCARCINOMA OF THE PROSTATE

Prostate specific antigen's rapid rise in prominence is due to its close association with Cancer of the Prostate (CaP). Whilst this is the most common cancer in Western men, its relatively slow growth allows many

men to `die with the disease rather than from it'. It is third to lung and large bowel cancer, in terms of male cancer deaths. In 1995 there were 8866 registered deaths in England and Wales from prostate cancer (Office for National Statistics, 1995).

The incidence has increased rapidly in recent years, which cannot be accounted for by better diagnosis of the disease alone (Chamberlain, 2000).

The prevalence of prostate cancer is proportional to age. The median age of affected men is around 72 years. Not all men with CaP develop invasive disease. Autopsy studies have shown foci of CaP in up to 50-70% of men over 70 years (Milford, 2001). However, only 25% of these cancers are larger than 1 cm3 in volume and likely to be significant (Murphy, 2000). Therefore, it appears that many men have microscopic foci of adenocarcinoma, which, paradoxically, never become clinically significant.

Geographically, Scandinavia has the highest worldwide incidence (Sweden: 75/100000) and the Far East the lowest (Japan: 8/100 000).

When racial factors are compared, however, by far the highest incidence is amongst black men in the USA (152/100 000) (Chamberlain *et al*., 2000). It appears that immigrants adopt the aetiological risks of their new country rather than retain those of their original country. Geographical trends have been used to try to establish aetiology for CaP. Pollutants, dietary factors,

sexual activity, venereal disease and hormonal factors have all been implicated in different studies (Giovannucci *et al.,* 2000). Unlike breast cancer, it is clear that familial CaP is less common, accounting for possibly only 10% of cases

(Carter *et al*., 1995). Pathologically, adenocarcinomas represent 95% of prostate cancers, the rest consisting of sarcomas, lymphomas, squamous cell carcinomas and metastases from other sites. The majority (80%) of prostate tumours originate in the peripheral zone of the prostate (Milford *et al*., 2001), the remaining few originating in the transition zone.

Prostate cancer is a multifocal disease in more than 85% of prostatectomy specimens, with 70% having bilateral disease (Milford *et al*., 2001). Prostatic intraepithelial neoplasia (PIN) is also found in many surgical specimens. This is characterized by cytologically atypical cells lining normal architecture ducts. Despite the close relationship between PIN and CaP, the absolute proof of PIN's natural history is lacking. However, when high-grade PIN alone is found in prostate biopsies, subsequent repeat biopsy shows CaP in 30-50%

(Brawer *et al*., 1997). As PIN does not lead to basement membrane disruption, serum PSA is unaffected.

Prognosis is proportional to the sum score (worse for high scores). After initially localized growth, the tumour penetrates the capsule along

perineural and lymphatic channels to reach the periprostatic tissues. The first metastases appear in the local obturator lymph nodes. Circulating tumour cells then lodge in the skeleton, forming osteosclerotic metastases. Rarely, metastases are found in the liver, bladder and adrenal glands.

With the introduction of PSA, the presentation of CaP to the clinician has changed markedly (Murphy, 2000). Previously, nearly all tumours presented at an advanced stage with either skeletal metastases or bladder outflow obstruction.

Currently in the UK, over one-third of patients diagnosed with CaP presented to the urologist with only an elevated PSA. At diagnosis, 25% are found to have organ-confined disease (Dearnaley, 1994). In the USA, where annual PSA screening has been advocated by the American Cancer Society, 60-75% of patients present to their urologist with organ-confined disease (Nash, 2000).

2.3 MALE SEX HORMONES

Hormones are natural substances made by the glands and organs of hormone system, occurring naturally in our body.

Testosterone is the major male androgen and is produced by the leydig cells in the male testis and in smaller amounts by the adrenal glands. It is responsible for male secondary sexual characteristics and sperm

production. The effects of low testosterone levels include low sex drive, changes in mood, loss of muscle and bone strength, and increase body fat (Finn, 2012). The amount of testosterone synthesized is regulated by the hypothalamic-pituitary-testicular axis. When testosterone levels are low, gonadotropin-releasing hormone (GnRH) is released by the hypothalamus which in turn stimulates the pituitary gland to release FSH and LH. These latter two hormones stimulate the testis to synthesize testosterone (Swerdkiff, 1992). This means that when the feedback mechanism is functioning properly, low testosterone level will induce secretion of high FSH and LH levels.

2.4 CALCIUM HOMEOSTASIS IN DIABETES MELLITUS

The vitamin D precursor 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) functions via genomic and non-genomic mechanisms in a numerous of cell types [9,34,53]. Moreover, the paracrine and autocrine modes of action of $1,25(OH)_2D_3$ appear to be important in several cell types, including adipocytes and secretory cells in the pancreas, duodenum and kidney [24,25,27,48]. Vitamin D, dietary precursor of $1, 25(OH)_2D_3$, is often considered important nutrient for maintaining good health for preventing diseases (Changhwan, *et al.*, 2017).

In addition, 1, $25(OH)_2D_3$ has an important role in the regulation of cellular $Ca2^+$ signaling, which is linked to cellular responses, signaling and secretion [46,47,50]. Sustained $Ca2^+$ signals triggered by 1, $25(OH)_2D_3$ have been researched for the regulation of apoptosis, a process that can determine cell death in diseases such as obesity and type 2 diabetes (T2DM) [5,36,46,47]. Moreover, $1,25(OH)^2D_3$-induced $Ca2^+$ signals ($Ca2^+$ oscillations) can regulate insulin secretion from pancreatic β-cells. Vitamin D status has been linked to insulin resistance and T2DM in observational studies (Changhwan, *et al.*, 2017).

Vitamin D deficiency and dysregulation of vitamin D metabolism have been associated with an increased risk of obesity and T2DM; however, the mechanism for an association between vitamin D and disease such as obesity and T2DM remains unclear (Satin *et al.*, 2015). In secretory cells, vitamin D has protective against apoptosis due to the transient and localized nature of the $Ca2^+$ signals induced by $1,25(OH)_2D_3$. Elucidation of the role of $1,25(OH)_2D_3$ in the regulation of cellular $Ca2^+$ signaling in obesity and T2DM may lead to the development of novel therapeutic and preventive modalities for these diseases. The purpose of this review is to discuss the roles of calcium in the regulation of insulin secretion and insulin resistance, with an emphasis on signaling pathways that involve vitamin D-dependent cellular $Ca2^+$ signaling (Satin *et al.*, 2015).

2.5 DIABETES:

Diabetes, often referred to by doctors as diabetes mellitus, describes a group of metabolic diseases in which the person has high blood glucose (blood sugar), either because insulin production is inadequate, or because the body's cells do not respond properly to insulin, or both. Patients with high blood sugar will typically experience polyuria (frequent urination), they

will become increasingly thirsty (polydipsia) and hungry (polyphagia) (Suresh, 2016).

Centre for Disease Control (CDC) (2018), describe Diabetes, as the condition in which the body does not properly process food for use as energy. Most of the food we eat is turned into glucose, or sugar, for our bodies to use for energy. The pancreas, an organ that lies near the stomach, makes a hormone called insulin to help glucose get into the cells of our bodies. When you have diabetes, your body either doesn't make enough insulin or can't use its own insulin as well as it should. This causes sugars to build up in your blood. This is why many people refer to diabetes as "sugar."

The meaning and origin of diabetes mellitus: Diabetes comes from Greek, and it means a "siphon". Aretus the Cappadocian, a Greek physician during the second century A.D., named the condition *diabainein*. He described patients who were passing too much water (polyuria) - like a siphon. The word became "diabetes" from the English adoption of the Medieval Latin diabetes. In 1675, Thomas Willis added mellitus to the term, although it is commonly referred to simply as diabetes. *Mel* in Latin means "honey"; the urine and blood of people with diabetes has excess glucose, and glucose is sweet like honey. Diabetes mellitus could literally mean "siphoning off sweet water". In ancient China people observed that ants would be attracted to some people's urine, because it was sweet. The term "Sweet Urine Disease" was coined (Suresh, 2016).

2.5.1DIABETES MELLITUS:

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.

The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of differentorgans, especially the eyes, kidneys, nerves, heart, and blood vessels (American Diabetes Association, 2010).

Several pathogenic processes are involved in the development of diabetes.

These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action (Shimazaki *et al.,* 2007).

Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia.

Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of uncontrolled

diabetes are hyperglycemia with ketoacidosis or the non-ketotic hyperosmolar syndrome.

Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction.

Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial, and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes.

The vast majority of cases of diabetes fall into two broad etiopathogenetic categories.

In one category, type 1 diabetes, the cause is an absolute deficiency of insulin secretion (Shimazaki *et al.,* 2007).

Individuals at increased risk of developing this type of diabetes can often be identified by serological evidence of an autoimmune pathologic process occurring in the pancreatic islets and by genetic markers. In the other, much more prevalent category, type 2 diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. In the latter category, a degree of hyperglycemia

sufficient to cause pathologic and functional changes in various target tissues, but without clinical symptoms, may be present for a long period of time before diabetes is detected. During this asymptomatic period, it is possible to demonstrate an abnormality in carbohydrate metabolism by measurement of plasma glucose in the fasting state or after a challenge with an oral glucose load.

The degree of hyperglycemia (if any) may change over time, depending on the extent of the underlying disease process. A disease process may be present but may not have progressed far enough to cause hyperglycemia. The same disease process can cause impaired fasting glucose (IFG) and/or impaired glucose tolerance

(IGT) without fulfilling the criteria for the diagnosis of diabetes. In some individuals with diabetes, adequate glycemic control can be achieved with weight reduction, exercise, and/or oral glucose lowering agents. These individuals therefore do not require insulin. Other individuals who have some residual insulin secretion but require exogenous insulin for adequate glycemic control can survive without it. Individuals with extensive-cell destruction and therefore no residual insulin secretion require insulin for survival. The severity of the metabolic abnormality can progress, regress, or stay the same. Thus, the degree of hyperglycemia reflects the severity of

the underlying metabolic process and its treatment more than the nature of the process itself (Geiss *et al.*, 2003).

2.5.2 CLASSIFICATION OF DIABETES MELLITUS AND OTHER CATEGORIES OF GLUCOSE REGULATION

Assigning a type of diabetes to an individual often depends on the circumstances present at the time of diagnosis, and many diabetic individuals do not easily fit into a single class. For example, a person with gestational diabetes mellitus (GDM) may continue to be hyperglycemic after delivery and may be determined to have, in fact, type 2 diabetes.

Alternatively, a person who acquires diabetes because of large doses of exogenous steroids may become normoglycemic once the glucocorticoids are discontinued, but then may develop diabetes many years later after recurrent episodes of pancreatitis. Another example would be a person treated with thiazides who develops diabetes years later. Because thiazides in themselves seldom cause severe hyperglycemia, such individuals probably have type 2 diabetes that is exacerbated by the drug. Thus, for the clinician and patient, it is less important to label the particular type of diabetes than it is to understand the pathogenesis of the hyperglycemia and to treat it effectively (American Diabetes Association, 2010).

Type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency)

Immune-mediated diabetes. This form of diabetes, which accounts for only 5–10% of those with diabetes, previously encompassed by the terms insulin-dependent diabetes, type 1 diabetes, or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the beta-cells of the pancreas. Markers of the immune destruction of the β -cell include islet cell autoantibodies, autoantibodies to insulin, autoantibodies to GAD (GAD65), and autoantibodies to the tyrosine phosphatases IA-2 and IA-2β. One and usually more of these autoantibodies are present in 85–90% of individuals when fasting hyperglycemia is initially detected. Also, the disease has strong HLA associations, with linkage to the DQA and DQB genes, and it is influenced by the DRB genes. These HLA-DR/DQ alleles can be either predisposing or protective. In this form of diabetes, the rate of β-cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others (mainly adults). Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or ketoacidosis in the presence of infection or other stress.

Still others, particularly adults, may retain residual β-cell function sufficient to prevent ketoacidosis for many years; such individuals eventually become dependent on insulin for survival and are at risk for ketoacidosis. At this latter stage of the disease, there is little or no insulin secretion, as

manifested by low or undetectable levels of plasma C-peptide. Immunemediated diabetes commonly occurs in childhood and adolescence, but it can occur at any age, even in the 8th and 9th decades of life. Autoimmune destruction of β-cells has multiple genetic predispositions and is also related to environmental factors that are still poorly defined. Although patients are rarely obese when they present with this type of diabetes, the presence of obesity is not incompatible with the diagnosis.

These patients are also prone to other autoimmune disorders such as

Graves' disease, Hashimoto's thyroiditis, Addison's disease, vitiligo, celiac sprue, autoimmune hepatitis, myasthenia gravis, and pernicious anemia(American Diabetes Association, 2010).

Idiopathic diabetes. Some forms of type 1 diabetes have no known etiologies.

Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity.

Although only a minority of patients with type 1 diabetes fall into this category, of those who do, most are of African or

Asian ancestry. Individuals with this form of diabetes suffer from episodic ketoacidosis and exhibit varying degrees of insulin deficiency between episodes. This form of diabetes is strongly inherited, lacks immunological evidence for β-cell autoimmunity, and is not HLA associated.

An absolute requirement for insulin replacement therapy in affected patients may come and go.

Type 2 diabetes (ranging from predominantly insulin resistance with relative insulin deficiency to predominantly an insulin secretory defect with insulin resistance)

This form of diabetes, which accounts for approximately 90–95% of those with diabetes, previously referred to as non–insulin-dependent diabetes, type 2 diabetes, or adult-onset diabetes, encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency At least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive. There are probably many different causes of this form of diabetes. Although the specific etiologies are not known, autoimmune destruction of β-cells does not occur, and patients do not have any of the other causes of diabetes listed above or below.

Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance. Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region. Ketoacidosis seldom occurs spontaneously in this type of diabetes; when seen, it usually arises in association with the stress of another illness such as infection. This form of

diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes.

Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications.

Whereas patients with this form of diabetes may have insulin levels that appear normal or elevated, the higher blood glucose levels in these diabetic patients would be expected to result in even higher insulin values had their β-cell function been normal. Thus, insulin secretion is defective in these patients and insufficient to compensate for insulin resistance. Insulin resistance may improve with weight reduction and/or pharmacological treatment of hyperglycemia but is seldom restored to normal. The risk of developing this form of diabetes increases with age, obesity, and lack of physical activity.

It occurs more frequently in women with prior GDM and in individuals with hypertension or dyslipidemia, and its frequency varies in different racial/ ethnic subgroups. It is often associated with a strong genetic predisposition, more so than is the autoimmune form of type 1 diabetes. However, the genetics of this form of diabetes are complex and not clearly defined.

Other specific types of diabetes:

Genetic defects of the β-cell.

Several forms of diabetes are associated with monogenetic defects in β-cell function. These forms of diabetes are frequently characterized by onset of hyperglycemia at an early age (generally before age 25 years). They are referred to as maturity-onset diabetes of the young (MODY) and are characterized by impaired insulin secretion with minimal or no defects in insulin action. They are inherited in an autosomal dominant pattern. Abnormalities at six genetic loci on different chromosomes have been identified to date.

The most common form is associated with mutations on chromosome 12 in a hepatic transcription factor referred to as hepatocyte nuclear factor (HNF)-1α.

A second form is associated with mutations in the glucokinase gene on chromosome 7p and results in a defective glucokinase molecule. Glucokinase converts glucose to glucose-6-phosphate, the metabolism of which, in turn, stimulates insulin secretion by the β-cell. Thus, glucokinase serves as the "glucose sensor" for the β-cell. Because of defects in the glucokinase gene, increased plasma levels of glucose are necessary to elicit normal levels of insulin secretion. The less common forms result from mutations in other transcription factors, including HNF-4β, HNF-1β, insulin promoter factor (IPF)1, and NeuroD1.

Point mutations in mitochondrial DNA have been found to be associated with diabetes and deafness. The most common mutation occurs at position

3,243 in the tRNA leucine gene, leading to an A-to-G transition. An identical lesion occurs in the MELAS syndrome (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like syndrome); however, diabetes is not part of this syndrome, suggesting different phenotypic expressions of this genetic lesion.

Genetic abnormalities that result in the inability to convert proinsulin to insulin have been identified in a few families, and such traits are inherited in an autosomal dominant pattern. The resultant glucose intolerance is mild. Similarly, the production of mutant insulin molecules with resultant impaired receptor binding has also been identified in a few families and is associated with an autosomal inheritance and only mildly impaired or even normal glucose metabolism.

Genetic defects in insulin action. There are unusual causes of diabetes that result from genetically determined abnormalities of insulin action. The metabolic abnormalities associated with mutations of the insulin receptor may range from hyperinsulinemia and modest hyperglycemia to severe diabetes. Some individuals with these mutations may have acanthosis nigricans. Women may be virilized and have enlarged, cystic ovaries. In the past, this syndrome was termed type A insulin resistance. Leprechaunism and the Rabson- Mendenhall syndrome are two pediatric syndromes that have mutations in the insulin receptor gene with

subsequent alterations in insulin receptor function and extreme insulin resistance. The former has characteristic facial features and is usually fatal in infancy, while the latter is associated with abnormalities of teeth and nails and pineal gland hyperplasia.

Alterations in the structure and function of the insulin receptor cannot be demonstrated in patients with insulin-resistant lipoatrophic diabetes. Therefore, it is assumed that the lesion(s) must reside in the postreceptor signal transduction pathways.

Diseases of the exocrine pancreas. Any process that diffusely injures the pancreas can cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatectomy, and pancreatic carcinoma. With the exception of that caused by cancer, damage to the pancreas must be extensive for diabetes to occur; adrenocarcinomas that involve only a small portion of the pancreas have been associated with diabetes. This implies a mechanism other than simple reduction in β-cell mass. If extensive enough, cystic fibrosis and hemochromatosis will also damage β-cells and impair insulin secretion. Fibrocalculous pancreatopathy may be accompanied by abdominal pain radiating to the back and pancreatic calcifications identified on X-ray examination.

Pancreatic fibrosis and calcium stones in the exocrine ducts have been found at autopsy.

Endocrinopathies:

Several hormones (e.g., growth hormone, cortisol, glucagon, and epinephrine) antagonize insulin action. Excess amounts of these hormones (e.g., acromegaly, Cushing's syndrome, glucagonoma, pheochromocytoma, respectively) can cause diabetes. This generally occurs in individuals with preexisting defects in insulin secretion, and hyperglycemia typically resolves when the hormone excess is resolved.

Somatostatinoma- and aldosteronoma-induced hypokalemia can cause diabetes, at least in part, by inhibiting insulin secretion. Hyperglycemia generally resolves after successful removal of the tumor.

Drug- or chemical-induced diabetes:

Many drugs can impair insulin secretion. These drugs may not cause diabetes by themselves, but they may precipitate diabetes in individuals with insulin resistance.

In such cases, the classification is unclear because the sequence or relative importance of β-cell dysfunction and insulin resistance is unknown. Certain toxins such as Vacor (a rat poison) and intravenous pentamidine can permanently destroy pancreatic β-cells. Such drug reactions fortunately

are rare. There are also many drugs and hormones that can impair insulin action. Examples include nicotinic acid and glucocorticoids.

Patients receiving β-interferon have been reported to develop diabetes associated with islet cell antibodies and, in certain instances, severe insulin deficiency.

Infections. Certain viruses have been associated with β-cell destruction. Diabetes occurs in patients with congenital rubella, although most of these patients have HLA and immune markers characteristic of type 1 diabetes. In addition, coxsackie virus B, cytomegalovirus, adenovirus, and mumps have been implicated in inducing certain cases of the disease.

Uncommon forms of immune-mediated diabetes:

In this category, there are two known conditions, and others are likely to occur. The stiff-man syndrome is an autoimmune disorder of the central nervous system characterized by stiffness of the axial muscles with painful spasms. Patients usually have high titers of the GAD autoantibodies, and approximately one-third will develop diabetes.

Anti-insulin receptor antibodies can cause diabetes by binding to the insulin receptor, thereby blocking the binding of insulin to its receptor in target tissues.

However, in some cases, these antibodies can act as an insulin agonist after binding to the receptor and can thereby cause hypoglycemia.

Anti-insulin receptor antibodies are occasionally found in patients with systemic lupus erythematosus and other autoimmune diseases. As in other states of extreme insulin resistance, patients with anti-insulin receptor antibodies often have acanthosis nigricans. In the past, this syndrome was termed type B insulin resistance (American Diabetes Association, 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Study Area and Participants

This study was conducted in Benin City. Benin City is located in Edo State, Nigeria and it is located at 6.34 latitude and 5.63 longitude. It is situated at an elevation of 88 meters above sea level. Benin City has a population of about 1,125,058 people, making it the biggest city in Edo State (Taboola, 2015). It is located in the South-South region of Nigeria. It shares boundaries with Ondo State (West), Delta State (South), and Kogi State (North).

The subjects for this study were clinically diagnosed adult male patients with prostate enlargement and adult males who had undergone prostatectomy in University of Benin teaching Hospital and Central Hospital in Benin City, and control subjects were recruited from apparently sex and age matched adult males within Benin City who had neither prostate enlargement nor undergone prostatectomy. A total of 88 participants were recruited for this study. The participants included both adult males with prostate enlargement (29 participants), adult males who had undergone prostatectomy (29 participants), and adult males who had neither prostate enlargement nor undergone

prostatectomy, as control (30 participants). Informed consent was obtained from each participant after proper notification and information on the nature of the research, risk involved, benefits as well as confidentiality by using a questionnaire.

3.2. Research Design

This is a randomized cross sectional case control study. The instrument for the collection was a well informed questionnaire. The questionnaire has two main sections i. e. the demographic variables such as age, sex, occupational status, place of origin, marital status, educational status and the basic health indices such as indication for use of medication and presence of any signs and symptoms of medical condition in the study area.

3.3. Inclusion Criteria

Only adult male patients already diagnosed with prostate enlargement or have already undergone prostatectomy, aged above 35years were enrolled in this study. They were age and sex matched with apparently healthy controls.

3.4. Exclusion Criteria

Males who were apparently healthy, aged below 35years and have no history of enlarged prostate gland were excluded from this study.

3.5. Ethical Approval

Ethical approval was sought from the research/ethical clearance committee of Ministry of Health, Benin City with reference number HM.1208/7458.

3.6. Sample Size Determination

The sample size for the study was obtained using the formular described by Daniel, 1999.

The sample size for this study will be determined based on three factors;

1. The estimated prevalence of variable interest from literature review.
2. Confidence interval of 95%.
3. The acceptable margin of error.

The sample size will be calculated according to the following formula:

$$N = \frac{t^2 \times p(1-p)}{m^2}$$

Where;

N = required sample size

t = confidence level interval at 95% (standard value of 1.96)

p = estimated prevalence of increased serum PSA level among males in Nigeria, 1.046 (Ikuerowo *et al*, 2013).

m = margin of error at 5% (standard value of 0.05)

$$N = \frac{1.96^2 \text{ x } 1.046\ (1 - 1.046)}{0.05^2}$$

$$N = \frac{3.8416 \times 1.046\ (-0.046)}{0.0025}$$

$$N = 73.9 \approx 74$$

$$N = 74$$

Minimum sample size = 74

Therefore, 58 test samples (29 male patients who have undergone prostatectomy and 29 male patients who have enlarged prostate gland but have not undergone prostatectomy) and 30 controls will be evaluated for this research.

3.7 Sample Collection

Under aseptic conditions, about 5 millimeters (5mls) of venous fasting blood sample was obtained from the antecubital vein of each subject using a sterile needle and syringe, and dispensed into a clean dry plain container. The sample in the plain container was left to clot undisturbed for 10minutes and centrifuged at 4000rpm for 10minutes to separate serum from the clot. The serum was dispensed into another clean and dry plain container. The serum samples were stored at -20°C until PSA, LH, FSH, prolactin, testosterone and progesterone were analysed.

3.8. Sample Analysis

3.8.1. Determination of Prostate Specific Antigen (PSA)

Laboratory method for assaying serum Prostate Specific Antigen (PSA)

Serum PSA level was determined by ELISA method from Calibiotech ELISA PSA kit, CA 92020, USA, according to manufacturer's instructions.

3.8.2. Estimation of Serum Testosterone Hormone

Laboratory method for assaying serum Testosterone

Serum Testosterone level was determined by ELISA method from Calibiotech ELISA Testosterone kit, CA 92020, USA, according to manufacturer's instructions.

3.8.3. Determination of Serum Leuteinizing hormone (LH)

Laboratory Method for assaying Luteinizing Hormone (LH)

Serum Luteinizing Hormone level was determined by ELISA method from Calibiotech ELISA LH kit, CA 92020, USA., according to manufacturer's instructions.

3.8.4. Estimation of Serum Follicle Stimulating Hormone (FSH)

Laboratory Method for assaying Follicle Stimulating Hormone (FSH)

Serum Follicle Stimulating Hormone level was determined by ELISA method from Calibiotech ELISA FSH kit, CA 92020, USA, according to manufacturer's instructions.

3.8.5. Determination of Serum Prolactin (PRL)

Laboratory Method for assaying Prolactin (PRL)

Serum Prolactin level was determined by ELISA method from Calibiotech ELISA PRL kit, CA 92020, USA, according to manufacturer's instructions.

3.8.6. Determination of Serum Progesterone

Laboratory Method for assaying Progesterone

Serum Progesterone level was determined by ELISA method from Calibiotech ELISA Progesterone kit, CA 92020, USA, according to manufacturer's instructions.

3.8.7. Estimation of Serum calcium

Laboratory Method for assaying serum calcium

Serum Calcium was determined by spectrophotometry method using Randox reagent from UK according to manufacturer's instructions.

3.9. Statistical Analysis

The data obtained were statistically evaluated using Statistical Package for Social Sciences Program (SPSS) version 16.0. The measured parameters were be expressed as mean and standard error of mean (SEM) for both tests and controls. The data generated were compared using Students' t-test and Analysis of Variance (ANOVA) at 95% confidence intervals and at 5% level of significance ($p < 0.05$). Correlation between parameters was done using correlation coefficient test at 5% level of significance (P-value < 0.05).

CHAPTER FOUR

4.0 RESULTS

Table 4.1: Statistical result showing age group, marital status of test and control

	Test	Control	χ^2	p
Age group				
Below 40	0(0.0)	11(36.7)	20.067	0.000
40-49	14(31.8)	7(23.3)		
50-59	18(40.9)	5(16.7)		
60 and above	12(27.3)	7(23.3)		
Marital Status				
Married	44(100.0)	23(76.7)	11.339	0.001
Single	0(0.0)	7(23.3)		

Table 4.2: Statistical result showing group of smokers, Drinkers, Time of first intercourse, frequency after first intercourse, enlightened diabetes, time of diagnosis and medication Feeling after diagnosis, Feeling after diagnosis, Stat of libido before diagnosis, frequency of sex after diagnosis, State of libido after diagnosis, frequently sex if libido is normal or high, Penile erection of test and control.

	Test	Control
Smoke		
Yes	3(6.8)	0(0.0)
No	41(93.2)	0(0.0)
Drink		
Yes	4(9.1)	0(0.0)
No	40(90.9)	0(0.0)
Time of first intercourse		
Never	0(0.0)	30(100.0)
After 20	40(90.9)	0(0.0)
Before 20	4(9.1)	0(0.0)
How frequent after 1st intercourse		
None	0(0.0)	30(100.0)
Weekly	44(100.0)	0(0.0)
Enlightened diabetes		
Yes	19(43.2)	0(0.0)
No	25(56.8)	0(0.0)

Time of diagnosis		
N/A	0(0.0)	30(100.0)
<1	5(11.4)	0(0.0)
>1	39(88.6)	0(0.0)
Medication		
Yes	33(75.0)	0(0.0)
No	11(25.0)	0(0.0)
Feeling after diagnosis		
Normal	35(79.5)	0(0.0)
Happy	9(20.5)	0(0.0)
Feeling after diagnosis		
Normal	21(47.7)	0(0.0)
Depressed	23(52.3)	0(0.0)
Stat of libido before diagnosis		
Normal	37(84.1)	0(0.0)
Low	7(15.9)	0(0.0)
How frequently do have sex after diagnosis		
Weekly	11(25.0)	0(0.0)
Occasionally	33(75.0)	0(0.0)
State of libido after diagnosis		
Normal	27(61.4)	0(0.0)
Low	17(38.6)	0(0.0)
How frequently do you have sex if libido normal or high		
Weekly	9(100.0)	0(0.0)
Occasionally	0(0.0)	0(0.0)
Penile erection		
Strong	21(47.7)	0(0.0)
Weak	23(52.)3)	0(0.0)

Table 4.3: Statistical result of PSA, FSH, LH, PROG, Testosterone and Calcium of test and control.

	Test	Control	$\chi 2$	p
PSA				
Normal	43(97.7)	29(96.7)	0.076	0.782
Abnormal	1(2.3)	1(3.3)		
FSH				
Normal	24(54.5)	16(53.3)	0.011	0.918
Abnormal	20(45.5)	14(46.7)		
LH				
Normal	31(70.5)	27(90.0)	4.021	0.045
Abnormal	13(29.5)	3(10.0)		
Prog				
Normal	7(15.9)	25(83.3)	33.040	0.000
Abnormal	37(84.1)	5(16.7)		
Testosterone				
Normal	25(56.8)	17(56.7)	0.000	0.990
Abnormal	19(43.2)	13(43.3)		
Calcium				
Normal	17(38.6)	20(66.7)	5.606	0.018
Abnormal	27(61.4)	10(33.3)		

Table 4.4: Statistical result showing PSA, FSH, LH, PRL, PROG, TESTO, CALCIUM of test and control.

	Test	Control	T	p
PSA (ng/ml)	0.72±0.98	1.37±7.50	-0.573	0.569
FSH (mIU/ml)	25.46±22.62	16.67±9.15	2.017	0.047
LH (mIU/ml)	5.66±4.42	5.56±2.78	0.106	0.916
PRL (ng/ml)	36.01±25.02	0.26±0.47	7.809	0.000
PROG (ng/ml)	0.14±0.20	0.11±0.13	0.646	0.520
TESTO (ng/ml)	3.04±2.55	5.10±3.93	2.744	0.008
CALCIUM (ng/ml)	6.93±2.85	8.37±0.61	-2.714	0.008

The above table 4.4, shows the various concentration of Progesterone, Follicle Stimulating Hormone, Luteinizing Hormone, Prolactin, Progesterone, Testosterone and Calcium. It shows the mean and standard error of mean of all the parameters and paired t-test of both test and control.

CHAPTER FIVE

DISCUSSION:

According to Centre for Disease Control (CDC), Diabetes is the condition in which the body does not properly process food for use as energy. The central aim of this study was to estimate the serum level of Progesterone, Follicle Stimulating Hormone, Luteinizing Hormone, Prolactin, Progesterone, Testosterone and Calcium in Adult diabetic males. It was observed that Prolactin (p=0.000) was increased in the test subjects than in the controls. This coincides with the work of Eva *et al.,* 1981, which reported an increase in prolactin in diabetic male. Prolactin a polypeptide also known as pituitary hormone has the ability to promote lactation in response to the suckling stimulus of hungry young mammals (Riddle *et al*., 1983). The functions of prolactin is related to reproduction, growth and development, metabolism, immune regulation, brain function and behavior (Freeman *et al*., 2000). Mooradian et al., 1990, has reported a significant higher serum prolactin level in Diabetes (Mooradian *et al.,* 1990). Studies have shown that prolactin level affect glucose metabolic regulation in both pregnant and non pregnant individuals (Ben-Jonathan *et al.,* 2010).

The result also shows significantly low testosterone level (p=0.008), significantly high FSH (p=0.04). Dhindsa et al., 2008, showed in her work that, men of same age have shown that the level of testosterone is lower in diabetic men as compared to non-diabetic men. Testosterone is the major male androgen

and is produced by the leydig cells in the male testis and in smaller amounts by the adrenal glands. It is responsible for male secondary sexual characteristics and sperm production. The effects of low testosterone levels include low sex drive, changes in mood, loss of muscle and bone strength, and increase body fat (Finn, 2012). The amount of testosterone synthesized is regulated by the hypothalamic-pituitary-testicular axis. When testosterone levels are low, gonadotropin-releasing hormone (GnRH) is released by the hypothalamus which in turn stimulates the pituitary gland to release FSH and LH. These latter two hormones stimulate the testis to synthesize testosterone (Swerdkiff et al., 1995). This means that when the feedback mechanism is functioning properly, low testosterone level will induce secretion of high FSH and LH levels. However, study carried out by Saudeep et al. has shown that both free and total testosterone levels are lower in men with diabetes than in non-diabetic men and their levels of pituitary hormones (FSH) was more lower than expected (Sanders et al., 2004). LH was insignificantly high ($p=0.916$). Progesterone ($p=0.520$) and PSA ($p=0.569$) were of no statistical significance.

Calcium ($p=0.008$) was significantly increased. This is in correlation with the research carried out by Abdulrehman *et al.*, 2019. Calcium is a major constituent of bones and teeth and also plays an essential role as second messenger in cell-signaling pathway. Calcium is required to maximize the attainment of peak bone mass during growth and to prevent the progressive

demineralization of bones later in life, which lead to osteoporosis, bone fragility, and an increased risk of fracture. Circulating calcium concentrations are tightly controlled by the parathyroid hormone (PTH) and vitamin D at the expense of the skeleton when dietary calcium intake are inadequate. PTH secretion in blood is stimulated by a decrease in ionized Ca2+ and conversely PTH secretion is stopped by an increase in ionized Ca2+. In the bone, PTH activates a process known as bone resorption, in which activated osteoclasts break down bone and subsequently release Ca2+ into the serum. Sultan *et al.,* have shown that the reduction in serum calcium level in diabetes mellitus is most probably due to hyperglycemia which increases calcium and phosphorus excretion in urine which is proportional to the degree of glucosuria, hypercalciuria by osmotic diuresis caused stimulation of bone resorption caused by secondary hyperparathyroidism. In response to urinary calcium loss, PTH secretion is mildly but significantly stimulated to maintain serum calcium concentrations. Excess urinary calcium seems to be derived from bone.

CONCLUSION

This study revealed decreased levels of testosterone, increased level of FSH with insignificantly high levels of LH which is suggestive of abnormal feedback mechanism in the hypothalamic-pituitary-testicular axis of these diabetic men.

The study also revealed that PSA and progesterone of test subjects were of no significant difference from that of the control.

Calcium significant decrease is most probably due to hyperglycemia which increases calcium and phosphorus excretion in urine.

RECOMMENDATION

We therefore, recommend further studies that will include HbA1c assay and sophisticated techniques in order to elucidate the possible underlying mechanisms involved in these abnormalities so that correct strategies for treatment can be employed.

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APPENDIX

Reagents used for prostate test:

Prostate Specific Antigen (PSA) 1ml

PSA Enzyme reagent 13ml

Streptavidin coated plate 96 wells

Substrate A 7ml

Substrate B 7ml

Stop solution 8ml

(Accubind ELISA MicrowellsMonobind Inc. Lake Forest, CA 92630, USA).

Principle

The important reagents needed for an immunoenzymometric assy include high infinity and specific antibodies (enzyme-labelled and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place at the surface of a microplate well through the interaction of streptavidin coated on the well and exogeneously added biotinylated monoclonal anti-PSA antibody. Upon mixing, a reaction results between the native antigen contained in serum, the monoclonal biotinylated antibody and the enzyme-labeled antibody, without competition or steric hindrance, to form a soluble sandwich complex. Simultaneously, the complex is deposited to the well through the high affinity reaction of

streptavidin and biotinylated antibody. After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity determined by a reaction with a substrate that produces light, in the antibody-bound fraction, is directly proportional to the native antigen concentration.

Reagent preparation

Working wash solution was prepared by adding 20ml of wash solution concentrate to 1000ml of deionized water and mixed well.

Test procedure:

- ❖ For each serum reference in the microplate wells, control and specimen to be examined was duplicated and formatted.
- ❖ 25µl of the appropriate serum reference, control or specimen was pipette into the assigned well.
- ❖ 100µl of PSA enzyme reagent was added to each well.
- ❖ For 20-30 seconds, the microplate was swirled gently for proper mixing.
- ❖ For 30 minutes at room temperature, they were covered and allowed to incubate.
- ❖ The microplate content was discarded by aspiration and the plate blotted dry.

- 0.350ml (350µl) of wash buffer was added and aspirated; this was done for additional two times.
- To all wells, 0.100ml (100µl) of working substrate solution was added.
- Incubation was done for 15minutes at room temperature.
- To each well, 0.050ml (50µl) of stop solution was added and mixing was done gently for 15-20 seconds.
- In each well, the absorbance was read at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) using a microplate reader (Dorfman and Shipley, 1965).

Calculation

- By using different serum references of known antigen values, a dose response curve was generated from which the antigen concentration of the unknown was ascertained.

Reagents used for testosterone test:

Testosterone calibrators (1ml/vial-Icons A-G)

Testosterone enzyme reagent (1.0ml/vial)

Steroid conjugate buffer (7.0ml/vial-Icon)

Testosterone biotin reagent (6.0ml-Icon)

Streptavidin coated plate (96 wells-Icon)

Wash solution concentrate (20ml-Icon)

Substrate A (7ml/vial-Icon S^A)

Substrate B (7ml/vial-Icon S^B)

Stop solution (8ml/vial-Icon)

(Accubind ELISA MicrowellsMonobind Inc. Lake Forest, CA 92630, USA).

Principle

The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate, enzyme-antigen conjugate and native antigen. Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competitive reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of insolubulized binding sites. After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration.

Reagent preparation

Working wash solution was prepared by adding 20ml of wash solution concentrate to 1000ml of deionized water and mixed well.

Test Procedure:

- ❖ For each serum reference, the microplate's wells, control and specimen to be examined was duplicated and formatted.

- The assigned well contained 0.010ml (10µl) of the appropriate serum reference, control or specimen.
- To all assigned wells, 0.050ml (50µl) of the working testosterone enzyme reagent was added.
- For 20-30 seconds, the microplate was swirled gently for proper mixing.
- For 60 minutes at room temperature, they were covered and allowed to incubate.
- The microplate content was discarded by aspiration and the plate blotted dry.
- 0.350ml (350µl) of wash buffer was added and aspirated; this was done for additional two times.
- To all wells, 0.100ml (100µl) of working substrate solution was added.
- Incubation was done for 15minutes at room temperature.
- To each well, 0.050ml (50µl) of stop solution was added and mixing was done gently for 15-20 seconds.
- In each well, the absorbance was read at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) using a microplate reader (Dorfman and Shipley, 1965).

Calculation

By using different serum references of known antigen values, a dose response curve was generated from which the antigen concentration of the unknown was ascertained.

Reagents Used for Luteinizing Hormone:

LH Calibrators (1ml/vial-Icons A-F),

LH Enzyme Reagent (13ml/vial-Icon), streptavidin coated plate (96 wells-Icons),

Wash solution concentrate (20ml/vial-Icon),

Substrate A (7ML/VIAL-Icon S^A),

Substrate B (7ml/vial-Icon S^B),

Stop solution (8ml/vial-Icon)

(Accubind ELISA Microwells Monobind Inc. Lake Forest, CA 92630, USA).

Principle:

The important reagents needed for an immunoenzymometric assay include high infinity and specific antibodies (enzyme-labelled and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-FSH antibody. Upon mixing, a reaction results between the native antigen contained in serum, the monoclonal

biotinylated antibody and the enzyme-labeled antibody, without competition or steric hindrance, to form a soluble sandwich complex. Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity determined by a reaction with a substrate that produces light, in the antibody-bound fraction, is directly proportional to the native antigen concentration.

Reagent Preparation:

All reagents were allowed to reach room temperature (18-25°C). Working wash solution was prepared by adding 20ml of wash solution concentrate to 1000ml of deionized water and mixed well.

Test Procedure:

- For each serum reference, the microplate's wells, control and specimen to be examined was duplicated and formatted.
- The assigned well contained 0.010ml (10µl) of the appropriate serum reference, control or specimen.
- To all assigned wells, 0.050ml (50µl) of the working LH enzyme reagent was added.
- For 20-30 seconds, the microplate was swirled gently for proper mixing.
- For 60 minutes at room temperature, they were covered and allowed to incubate.

- ❖ The microplate content was discarded by aspiration and the plate blotted dry.
- ❖ 0.350ml (350µl) of wash buffer was added and aspirated; this was done for additional two times.
- ❖ To all wells, 0.100ml (100µl) of working substrate solution was added.
- ❖ Incubation was done for 15minutes at room temperature.
- ❖ To each well, 0.050ml (50µl) of stop solution was added and mixing was done gently for 15-20seconds.
- ❖ In each well, the absorbance was read at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) using a microplate reader (Danzer and Braunstein, 1980, Kosasa, 1981).

Calculation:

By using different serum references of known antigen values, a dose response curve was generated from which the antigen concentration of the unknown was ascertained.

Reagents Used for Follicle Stimulating Hormone:

FSH Calibrator (1ml/vial-Icon A-F)   0, 5, 10, 25, 50, and 100mIU/Ml.

FSH Enzyme Reagent (13ml/vial-Icon)

Streptavidin coated plate (96 wells- Icon)

Wash solution concentrates (20ml-Icon)

Substrate A: tetramethylbenzidine solution in buffer (7.0ml/vial-Icon S^A)

Substrate B: hydrogen peroxide in buffer (7.0ml/vial-Icon S^B)

Stop solution (8ml/vial-Icon)

(Accubind ELISA MicrowellsMonobind Inc. Lake Forest, CA 92630, USA).

Principle

This is based on the principle of a solid phase enzyme-linked immunosorbent assy. In this assay procedure, the immobilization takes place at the surface of a microplate well through the interaction of streptavidin coated on the well and exogeneously added biotinylated monoclonal anti-FSH antibody. Upon mixing, a reaction results between the native antigen contained in serum, the monoclonal biotinylated antibody and the enzyme-labeled antibody, without competition or steric hindrance, to form a soluble sandwich complex. Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration.

Reagent Preparation

All reagents were allowed to reach room temperature (18-25°C). Working wash solution was prepared by adding 20ml of wash solution concentrate to 1000ml of deionized water and mixed well.

Test Procedure:

- For each serum reference, the microplate's wells, control and specimen to be examined was duplicated and formatted.
- The assigned well contained 0.050ml (50µl) of the appropriate serum reference, control or specimen.
- To all assigned wells, 0.100ml (100µl) of the working FSH enzyme reagent was added.
- For 20-30 seconds, the microplate was swirled gently for proper mixing.
- For 60 minutes at room temperature, they were covered and allowed to incubate.
- The microplate content was discarded by aspiration and the plate blotted dry.
- 0.350ml (350µl) of wash buffer was added and aspirated; this was done for additional two times.
- To all wells, 0.100ml (100µl) of working substrate solution was added.
- Incubation was done for 15minutesat room temperature.
- To each well, 0.050ml (50µl) of stop solution was added and mixing was done gently for 15-20seconds.
- In each well, the absorbance was read at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) using a microplate reader (Odell and Parlow 1981).

Calculation

By using different serum references of known antigen values, a dose response curve was generated from which the antigen concentration of the unknown was ascertained.

Reagents Used Serum Prolactin Test:

PRL Calibrator (1ml/vial-Icon A-F)

PRL Enzyme Reagent (13ml/vial-Icon)

Streptavidin coated plate (96 wells- Icon)

Wash solution concentrate (20ml-Icon)

Substrate A (7.0ml/vial-Icon S^A)

Substrate B (7.0ml/vial-Icon S^B)

Stop solution (8ml/vial-Icon)

(Accubind ELISA MicrowellsMonobind Inc. Lake Forest, CA 92630, USA).

Reference range: 3.8-23.2ng/ml

Principle

The important reagents needed for an immunoenzymometric assy include high infinity and specific antibodies (enzyme-labelled and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place at the surface of a microplate well through the interaction of streptavidin coated on the well and exogeneously added biotinylated monoclonal anti-PRL antibody. Upon mixing, a reaction

results between the native antigen contained in serum, the monoclonal biotinylated antibody and the enzyme-labeled antibody, without competition or steric hindrance, to form a soluble sandwich complex. Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity determined by a reaction with a substrate that produces light, in the antibody-bound fraction, is directly proportional to the native antigen concentration.

Reagent preparation

All reagents were allowed to reach room temperature (18-25°C). The reagents were mixed properly by gentle inversion before use.

Test Procedure:

- ❖ For each serum reference, the microplate's wells, control and specimen to be examined was duplicated and formatted.
- ❖ The assigned well contained 0.025ml (25µl) of the appropriate serum reference, control or specimen.
- ❖ To all assigned wells, 0.100ml (100µl) of the working PRL enzyme reagent was added.

- For 20-30 seconds, the microplate was swirled gently for proper mixing.
- For 60 minutes at room temperature, they were covered and allowed to incubate.
- The microplate content was discarded by aspiration and the plate blotted dry.
- 0.350ml (350µl) of wash buffer was added and aspirated; this was done for additional two times.
- To all wells, 0.100ml (100µl) of working substrate solution was added.
- Incubation was done for 15 minutes at room temperature.
- To each well, 0.050ml (50µl) of stop solution was added and mixing was done gently for 15-20 seconds.
- In each well, the absorbance was read at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) using a microplate reader (Aufrere and Benson, 1976).

Calculation

By using different serum references of known antigen values, a dose response curve was generated from which the antigen concentration of the unknown was ascertained.

Reagents Used for Serum Progesterone:

Progesterone Calibrator (1ml/vial-Icon A-G)

Progesterone Enzyme Reagent (6ml/vial-Icon)

Progesterone Biotin Reagent (6ml/vial-Icon)

Streptavidin Coated Plate (96 wells- Icon)

Wash Solution Concentrate (20ml-Icon)

SubstrateReagent (12ml/vial-Icon SN)

Stop Solution (8ml/vial-Icon)

(Accubind ELISA MicrowellsMonobind Inc. Lake Forest, CA 92630, USA).

Principle

The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing native antigen, a competitive reaction occurs between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. A simultaneous reaction occurs between the biotin attached to the antibody and the streptavidin immobilized on the microwell. This effects the separation of the antibody bound fraction after decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration.

Reagent Preparation

All reagents were allowed to reach room temperature (18-25°C). the reagents were mixed properly by gentle inversion before use.

Test Procedure:

- For each serum reference, the microplate's wells, control and specimen to be examined was duplicated and formatted.
- The assigned well contained 0.025ml (25µl) of the appropriate serum reference, control or specimen.
- To all assigned wells, 0.050ml (50µl) of the progesterone biotin reagent was added.
- For 20-30 seconds, the microplate was swirled gently for proper mixing.
- For 60 minutes at room temperature, they were covered and allowed to incubate.
- The microplate content was discarded by aspiration and the plate blotted dry.
- 0.350ml (350µl) of wash buffer was added and aspirated; this was done for additional two times.
- To all wells, 0.100ml (100µl) of working substrate solution was added.
- Incubation was done for 15 minutes at room temperature.
- To each well, 0.050ml (50µl) of stop solution was added and mixing was done gently for 15-20seconds.
- In each well, the absorbance was read at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) using a microplate reader (Abraham, 1981).

Calculation

By using different serum references of known antigen values, a dose response curve was generated from which the antigen concentration of the unknown was ascertained.

Principle of Test for Serum Calcium Test:

This procedure is based on the reaction of calcium ions (Ca^{++}) with 0-cresophthalein complex in alkaline solution to form an intense violet coloured complex which shows maximum absorbance at 578nm.

Working Reagent

Reagent 1 and 2 (mixed in the ratio 1:1)

Calcium standard

Calcium standard concentration 10mg/dl

Laboratory procedure

- ❖ To test tubes labeled blank, standard and sample, 1000µL of working reagent was dispensed respectively.
- ❖ 10µL of standard solution was placed into test tube labeled standard while 10µL of sample was placed into test tubes labeled sample and 10µL of distilled water into test tube labeled blank.
- ❖ Sample were mixed and incubated at room temperature for 5minutes.

- Absorbance of standard and sample were read at 578nm against reagent blank.