RELATIONSHIP BETWEEN SERUM FASTING INSULIN CONCENTRATION AND OTHER MARKERS OF DIABETIC CONTROL IN ZARIA

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FACULTY OF MEDICINE

AHMADU BELLO UNIVERSITY,

ZARIA

MAY, 2015

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BY

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M.Sc/MED/2009-2010/0791

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES,

AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE WARD OF A MASTER DEGREE IN CHEMICAL PATHOLOGY.

DEPARTMENT OF CHEMICAL PATHOLOGY,

FACULTY OF MEDICINE

AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

MAY, 2015

DECLARATION

I hereby declare that the work in this thesis entitled **RELATIONSHIP BETWEEN SERUM FASTING INSULIN CONCENTRATION AND OTHER MARKERS OF DIABETIC CONTROL IN ZARIA** has been carried out by me in the department of Chemical Pathology Ahmadu Bello University Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis waspreviously presented for another degree or diploma at this or any other institution.

Mohammed El-Bashir JIBRIN _____ _____

(Signature) (Date)

CERTIFICATION

The thesis entitled **RELATIONSHIP BETWEEN SERUM FASTING INSULIN CONCENTRATION AND OTHER MARKERS OF DIABETIC CONTROL IN ZARIA** byMOHAMMED EL-BASHIR JIBRIL meets the regulations governing the award of the degree of master degree in chemical pathology of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ACKNOWLEDGEMENT

All praises and gratitude be to Almighty God who has given me wisdom, good health and strength to carry out this study.

My sincere appreciation goes to my supervisors, Professor P.O Anaja, Dr S.A Akuyam and Dr Fatima Bello whose constructive criticisms, constant advice, guidance and patience saw me through this work. Appreciation also goes toDrRabiuAdamu, Dr Mohammed Manu, DrAminaDogara, Dr. R. Yusuf, DrAbdulazeez Hassan, Dr A M Suleiman, DrAbdullahi Mohammed, Professor I.S Aliyu, Professor A I Mamman,Dr S M Aminu and for their professional advice, encouragement and support.

Special appreciation goes to my parents AlhajiJibril Mohammed Galadanchi (late) and HajiaBilkisuJibril Mohammed for my proper upbringing and prayers. My warmest appreciation also goes to my beloved wife Barrister Binta Musa Mohammed, my children AbubakarSadiq El-Bashir, Bilkis Noor El-Bashir for their understanding and support during the period of the research work.

I also wish to acknowledge the immense support and prayers by my brothers and sisters, EngrAbubakarJibril Mohammed, MalamKabiruJibril, Barrister Mohammed Jibril Mohammed, MalamYahyaJibril, Engineer (Dr.) Mohammed Jibril, NuraJibril, Engineer Mustapha Jibril, Baba Balin, JamilaJibril, Ibrahim Jibril, Fatima Jibril, EngrAishatuJibril Mohammed, AminatuJibril Mohammed, Engr Umar Jibril Mohammed, Ahmed Jibril, Faisal Jibril, Suleiman Jibril, AbdullahiJibril, HindatuJibril, Halima Jibril, Sa'adatu Abbas, Amina Mohammed, Fatiti Ibrahim, Rahila Mustapha and Rabi Kabiru. May the almighty God bless you and your families.

To my colleagues, resident doctors of Chemical Pathology department, other doctors and nurses who assisted me during the study especially Dr (Mrs.) H M Suleiman, and other residents and friends too numerous to mention, I say thank you all. I also want to extend my sincere appreciation to Ann Achinze, Muhammad AuwalAbubakar, Umar Farouk Abubakar, Suwidi Suleiman, Shuaibu Ibrahim (maiunguwa), Abdul Aminu, Zainab Isa, JimmaiAliyu, Sa'adatuShehu, Sa'adiyya Y M Bulangu and Malam Yusuf Mohammed Bello.

ABSTRACT

Diabetes mellitus is a common disease associated with insulin deficiency or resistance. Most studies on diabetes focus on glucose, HbA1c and only a few on fructosamine. Insulin assay was never considered as part of the management of diabetes mellitus. The study was therefore carried out to relate serum fasting insulin level with other markers of glycaemic control in type 2 diabetes. Eighty eight diabetic patients and 88 non diabetic healthy controls were enrolled into the study. Blood specimens from these volunteers were collected and analyzed for insulin and HbA1c using ELISA and chromatographic methods respectively while glucose and fructosamine were analyzed using spectrophotometric methods. Weight, height and blood pressure were also taken and body mass index calculated. Statistical analysis was carried out using Epi Info. Serum fasting insulin and other markers of glycaemic control were higher in diabetic patients than innon diabetic healthy controls. There is significant correlation between the serum fasting insulin with body mass index in diabetic patients ($p = 0.0291$) but not in non diabetic healthy controls ($p = 0.1341$). There was no correlation between other markers of glycaemic control and BMI in both diabetics and controls ($p>0.05$). Serum fasting insulin level correlated positively and significantly with glucose ($p=0.0000$, $r= + 0.73$), HbA1c ($p=0.0000$, $r= +0.69$) and fructosamine ($p=0.0000$, $r= + 0.69$). Insulin measurement may be a useful adjunct in the biochemical evaluation of diabetic Nigerians.

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ABBREVIATIONS USED

ANOVA	Analysis of variance
ADA	American Diabetic Association
BP	Blood pressure
d	Precision
ELISA	Enzyme Linked Immunosorbent Assay
Kg	Kilogram
Km	Kilometer
Km/h	Kilometer per hour
mm	millimetre
mmHg	Millimeters of mercury
M	Meter
NPHCDA	National Primary Health Care Development Agency
NAFDAC	National Agency for Food and Drug Administration Control
n	Sample size or frequency
nm	Nanometer
ng/mL	Nanogram per millilitre
P	Prevalence
pg/mL	picogram per millilitre
rpm	Revolutions per minutes
SD	Standard deviation
SON	Standard Organisation of Nigeria
U.S	United States
WHO	World Health Organisation
z	Confidence interval
°	Degree

%	Per cent
°C	Degree Celsius
µIU/L	Micro international unit per liter
µg/L	Micro gram per litre

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Diabetes mellitus (DM) is a group of metabolic disorders of carbohydrate metabolism in which glucose is underutilized leading to hyperglycaemia. Elevation of blood glucose concentration is secondary to either resistance to the action of insulin, insufficient insulin secretion, or both (Ezenwaka*et al*, 2003; Tilahun*et al*, 2007). Diabetes mellitus is a common disease. Recent estimates indicate that, there were 171 million people in the world with DM in the year 2000 and this was projected to increase to 366 million by 2030 (WHO, 2000). Also, there were over one million and seven hundred thousand Nigerians who lived with DM in the year 2000 (Chijioke*et al*, 2010), and was projected to slightly over 4.8 million population by 2030 (WHO, 2000). Local prevalence of DM in Ibadan, Nigeria was 2.8 % (Owaoje et al, 1997). Prevalence of DM among general Nigerian population has increased by almost 1 % in two years, from 3.9 % in 2010 to 4.83 % in 2012 (IDF, 2010; IDF, 2012). World Health Organization reported that in the year 2000 over seven million diabetics lived in Africa and one million seven hundred thousand (24 %) were Nigerians (Chijioke*et al*, 2010). By inference, about 1.4 % of the Nigerian population in the year 2000 irrespective of age and sex were diabetics. Prevalences of 1.6 %, 2.8 % and 3.9 % were observed in Ibadan, Zaria and in Nigerian general population respectively (Owaoje*et al*, 1997; Bakari*et al*, 2004; IDF, 2010). In 2012, the prevalence in Nigeria increased to 4.83 % (IDF, 2012) and 4.99 % in 2013 (IDF, 2013). About 8.3 % of the United States' total population and 26.9 % of U.S residents aged 65 years and older are diabetics (National Diabetes Mellitus fact sheet, 2011; National Diabetes Mellitus statistics, 2011).

Over the past 30 years the prevalence of DM has been increasing steadily (Chijioke*et al*, 2010). A hospital survey in Ibadan estimated a prevalence of 0.4% (Osuntokun*et al*, 1971),

while screening for DM during a World DM Day (November 14) in Lagos metropolis a prevalence of undiscovered DM of 1.6% was found (Ohwovoriole*et al*, 1989). A national survey in 1992 by the Non-communicable Disease Expert Committee of the Federal Ministry of Health recorded a prevalence of 2.2%. A survey by Puepet (1994) in urban adults in Jos metropolis discovered a prevalence of undiscovered DM to be 3.1%. The progressive increase in the prevalence rates of DM is associated with lifestyle changes (Curtis *et al*, 2005); overweight and obesity, physical inactivity, alcohol consumption, dietary changes and cigarette smoking- factors that are potentially modifiable (Bakari et al, 2004; SamreenRiaz et al, 2009). Unfortunately, most Nigerians are ignorant of DM and healthy lifestyle (Bakari et al, 2004).

The vast majority of diabetic patients are classified into one of two broad categories: type 1 DM, which is caused by an absolute deficiency of insulin, and type 2 DM, which is characterized by the presence of insulin resistance with an inadequate compensatory increase in insulin secretion. In addition, women who develop DM during their pregnancy are classified as having gestational diabetes mellitus (GDM). Finally, there are a variety of uncommon and diverse types of DM, which are caused by infections, drugs (Bakari*et al*, 2007), endocrinopathies, pancreatic destruction and genetic defects. These unrelated forms of DM are included in the "Other Specific Types" and classified separately (Crook, 2006; Afonja, 2011).

Patients with diabetes mellitus usually complain of polyuria, polydipsia, polyphagia and unexplained loss of weight (Sonny *et al*., 2011). The fasting plasma glucose in an adult is usually equal or less than 5.1 mmol/L (Afonja*et al*, 2011). Diabetes mellitus is diagnosed when fasting plasma glucose is 7.0 mmol/L and above on more than one occasion. Also, DM can be diagnosed when random blood glucose is 11.1 mmol/L or more on more than one occasion. When there are clear signs and symptoms of DM in a patient, a single random

blood glucose value of 11.1 mmol/l can diagnose DM. Any of the three criteria above confirms the diagnosis (Crook *et.al*, 2006; Margullies*et al*, 2010; Afonja, *et.al* 2011).

Oral glucose tolerance test (OGTT) is done when there is a high index of clinical suspicion of DM such as a patient at risk of gestational diabetes mellitus with equivocal blood glucose (Olarinoye*et al*, 2004; Lin, 2012). When fasting blood glucose is between 5.5 – 7.0 mmol/L or random blood glucose between 7.0 – 11.1 mmol/L on more than one occasion, OGTT can also be performed (Crook *et al*, 2006, Margullies*et al*, 2010; Afonja*et al*, 2011).

Measurement of serum or plasma glucose is the key in the diagnosis of DM. Recently American Diabetes Association included HbA1c of 6.5 % or more as an additional diagnostic criterion. The American Association of Clinical Endocrinologists, however, recommends that HbA1c be considered as an additional optional diagnostic criterion, rather than a primary criterion for diagnosis of DM. Previously, HbA1c was only recommended as a follow up investigation done every three months in diabetic patients. Other biochemical investigations are serum fructosamine, serum fasting lipid profile to assess cardiovascular complications, renal function tests and microalbuminuria to assess the kidney status and urinalysis (Crook *et al*, 2006).

Diabetes mellitus is associated with many complications such as coronary artery disease, cerebrovascular accidents, retinopathy, blindness, hypertension, kidney diseases, neuropathy, amputations, pregnancy complications, dental diseases and psychiatric diseases (Chijioke*et al*, 2010; National Diabetes Mellitus Statistics, 2011; Sonny *et al*., 2011).

Diabetes mellitus accounts for 60 % and 75 % of all non traumatic amputations of lower limbs in United States and Western Europe respectively (Ogirima*et al*, 2000). Also 44 % of all new cases of renal failure in 2008 and 67 % of all cases of hypertension occur among diabetic patients in United State (National Diabetes Mellitus Statistics, 2011). Risk of stroke was 2 to 4 times higher among diabetic patients in United States. In fact, DM is the seventh

overall leading cause of death in the United States (National Diabetes Mellitus Statistics, 2011).

A ten years retrospective study done in Ilorin, Nigeria revealed several complications among diabetic patients which include hyperglycaemic emergencies (29.81%), septicaemia (21.91 %), diabetic foot syndrome (16.81 %), stroke (8.28 %), hypoglycaemia (6.75 %), nephropathy(5.98 %), neuropathy and retinopathy with the overall mortality rate of 32.5 % (Chijioke A. *et al,* 2010). Another study in University of Port-Harcourt Teaching Hospital, Nigeria revealed that diabetes mellitus accounts for 10.4 % of all medical admissions from 1994 to 2005 and mortality due to diabetes was 17.2 % (Unachukwu*et al*, 2008). Studies from Zaria reported that diabetes accounts for 5.3 % of major amputations (Ogirima et al, 2000). Thirty six per cent of diabetics in Kano had features of diabetic retinopathy (Lawal*et al*, 2012).In neonates and pregnant mothers, its associated complications are macrosomia, cardiac defects, abortions, polyhydramnios, pre-eclampsia, eclampsia, maternal hypertension, premature labour and haemorrhages (Abourawi, 2006; Crook *et al*, 2006).

However, there is limited data in Nigeria concerning the pattern of insulin among Nigerian diabetics. Therefore, this study aims at studying fasting insulin concentration among diabetic patients and controls and how it relates with other markers of glycaemic controls. It is hoped that this will give more insight into the pathophysiology of DM and assess compliance of the patients to the treatment modalities.

1.2 STATEMENT OF THE PROBLEM

Prevalence of diabetes mellitus and its related complications are on the increase all over the world. Mortality is still high and its complications remain fatal. The cost of management of diabetes mellitus is enormous. Diabetes mellitus is an insulin deficiency or resistance disorder. Some diabetic patients have complete or partial deficiency of insulin

while some only developed resistance and yet insulin is not considered either as a marker in the diagnosis or follow up of this condition.

1.3 JUSTIFICATION OF THE STUDY

There is paucity of data on serum insulin levels in relation to indices of glycaemic control in our environment. There is need to know serum levels of insulin in diabetic patients and controls. It is hoped that this knowledge will give more insight into the pathophysiology of diabetes mellitus and assess compliance of the patients to the treatment modalities or effect of treatment on insulin secretion and action.

1.4 General Objective

To evaluate the relationship between serum fasting insulin levels and markers of glycaemic control among diabetic patients in Ahmadu Bello University Teaching Hospital (ABUTH), Zaria.

1.5 Specific Objectives:

1. To measure the serum levels of fasting insulin, glucose, fructosamine and HbA1c in diabetic patients and healthy controls.
2. To relate serum fasting insulin, glucose, fructosamine and HbA1c levels with body mass index (BMI) in diabetic patients and healthy controls.
3. To compare serum fasting insulin concentration with fasting plasma glucose concentration, fructosamine and HbA1c in diabetic patients.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 CARBOHYDRATES

Carbohydrates are aldehyde or ketone derivatives of polyhydroxy (more than one —OH group), alcohols, or compounds that yield these derivatives on hydrolysis. Carbohydrates are compounds containing C, H, and O. The general formula for a carbohydrate is Cx(H2O)y. There are some derivatives from this basic formula because carbohydrate derivatives can be formed by the addition of other chemical groups, such as phosphates, sulfates, and amines. The classification of carbohydrates is based on four different properties: (a) the size of the base carbon chain, (b) the location of the CO function group, (c) the number of sugar units, and (d) the stereochemistry of the compound. Carbohydrates can be grouped into generic classifications based on the number of carbons in the molecule. For example, triosescontain three carbons, tetroses contain four, pentoses contain five, and hexoses contain six. In actual practice, the smallest carbohydrate is glyceraldehyde, a three-carbon compound (Freeman *etal*, 2013)

Carbohydrates perform numerous and significant functions in the body, ranging from being structural components of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (*ribose* and *deoxyribose*sugars) to serving as sources of energy *(glucose).* Glucose is derived from the breakdown of carbohydrates in the diet (grains, starchy vegetables, and legumes) and in body stores (glycogen), and by endogenous synthesis from protein or from the glycerol moiety of triglycerides. When energy intake exceeds expenditure, the excess is converted into glycogen and fat for storage in liver or muscle and adipose tissue respectively. When energy expenditure exceeds calorie intake, endogenous glucose formation occurs from the breakdown of carbohydrate stores and from non-carbohydrate sources (e.g., amino acids, lactate, glycerol) (Freeman *etal*, 2010).

Disorders involving carbohydrates metabolism are divided into groups; hyperglycaemia and hypoglycaemia. Hyperglycaemia relates closely with diabetes mellitus. It occurs when blood glucose concentration exceeds the upper reference value of a population (Chijioke*et al*; 2010). Hypoglycaemia on the other hand is when glucose concentration is below the lower reference value of a population generally accepted as less than 2.8 mmol/L (Chijioke*et al*, 2010; Freeman *etal*, 2010; Afonja, *et.al* 2011). Early detection of diabetes mellitus is the aim of the American Diabetes Association (ADA) guidelines established in 1997 (ADA, 1997). Acute and chronic complications may be minimized with proper diagnosis, monitoring, and treatment. The laboratory plays an important role through periodic measurements of glycated haemoglobin and microalbuminuria (Freeman *etal*, 2010).

2.2 DIABETES MELLITUS

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It has been defined by the World Health Organization (WHO), on the basis of laboratory findings, as a fasting venous plasma glucose concentration of 7.0 mmol/L or more (on more than one occasion or once in the presence of diabetes symptoms) or a random venous plasma glucose concentration of 11.1 mmol/L or more. Sometimes an oral glucose tolerance test (OGTT) may be required to establish the diagnosis in equivocal cases (Olarinoye*et al*, 2004; Crook *et al*, 2006, Margullies*et al*, 2010; Afonja*et al*, 2011).

2.2.1 Classification of Diabetes Mellitus

In the year 1979, National Diabetes Data Group developed a classification and diagnosis scheme for diabetes mellitus. This scheme included dividing diabetes into two broad categories: type 1, insulin-dependent diabetes mellitus (IDDM); and type 2, non–insulin-dependent diabetes mellitus (NIDDM). Established in 1995, the International Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, working under the

sponsorship of the American Diabetes Association, was given the task of updating the 1979 classification system. The proposed changes included eliminating the older terms of IDDM and NIDDM. The categories of type 1 and type 2 were retained, with the adoption of Arabic numerals instead of Roman numerals ((Freeman *etal*, 2013).

Therefore, the American Diabetes Association/ World Health Organization (WHO) guidelines recommend the following categories of diabetes:

1. Type 1 diabetes
2. Type 2 diabetes
3. Other specific types of diabetes
4. Gestational diabetes mellitus (GDM)

2.2.1.1 Type 1 Diabetes Mellitus

Type 1 DM results from autoimmune destruction of the pancreatic β-cells (Cai*etal*, 2007). It is previously called insulin-dependent DM. Markers of immune destruction of the β-cell are present at the time of diagnosis in 90% of individuals and include antibodies to the islet cell (ICAs) which are mainly IgG or rarely IgA/ IgM, glutamic acid decarboxylase (GAD 65), and insulin auto-antibodies (IAAs), tyrosine phosphatases i.e. insulinoma associated antigens (1A – 2A) and 1A2bA (Afonja et al 2011; Aaron et al, 2011; Freeman *etal*, 2013). Most of these cases are due to immune mediated processes and may be associated with other autoimmune disorders such as Addison's disease, vitiligo and Hashimato'sthyroditis. It has been postulated that many cases follow a viral infection that has damaged the β-cells of the pancreatic islets such as congenital rubella, coxsakie B virus, echo and maternal enteroviruses (Afonja*et al* 2011). The beta cells of the islet may be destroyed directly by the virus or may be selectively destroyed by a chronic mononuclear cell inflammation produced by the antibody reaction on the surface of the cell. Development of antibodies due to a virus, drug or foreign protein as is found in cow's milk has a chronic and

insidious rather than acute onset (Afonja*et al* 2011). Those at risk of immune mediated type 1 DM include HLA-types DR3 and DR4 of the major histocompatibility complex. There is also genetic susceptibility to the development of Type 1 diabetes mellitus. This susceptibility is associated with HLA on chromosome 6 as well as other genetic factors such as the insulin gene on chromosome 11 and the T-lymphocyte antigen on chromosome 2 (Afonja*et al*, 2011). While this form of DM usually occurs in children and adolescents, it can occur at any age. Younger individuals typically have a rapid rate of β-cell destruction and present with ketoacidosis, while adults often maintain sufficient insulin secretion to prevent ketoacidosis for many years. There is another form of type 1 DM called idiopathic DM that is not autoimmune mediated but is strongly inherited. This is commoner among Africans and Asians (Crook *et al*, 2006; Margullies*et al,* 2010; Afonja*et al*, 2011).

Type 1 constitutes only less than 5 % of all cases of diabetes mellitus. Characteristics of type 1diabetes include abrupt onset, insulin dependence and ketosis tendency. Signs and symptoms include polydipsia (excessive thirst), polyphagia (increased food intake), polyuria (excessive urine production), rapid weight loss, hyperventilation, mental confusion, and possible loss of consciousness (due to increased glucose to brain). Complications are either divided into acute and chronic or microvascular and macrovascular. These include problems like nephropathy, neuropathy, retinopathy, coronary artery disease, cerebrovascular accident, dementia, diabetic foot and gastropathy. Increased heart disease is also found in patients with diabetes (Sonny *et al*., 2011). *Idiopathic type 1diabetes* is a form of type 1 diabetes that has no known etiology, is strongly inherited, and does not have β-cell autoimmunity. Individuals with this form of diabetes have episodic requirements for insulin replacement.

2.2.1.2 Type 2 Diabetes Mellitus

Type 2 diabetes mellitus was previously called non-insulin dependent DM (Crook *et al*, 2006). Glucose production by the liver exceeds glucose utilization in the tissues and fasting hyperglycaemia results. It is characterized by insulin resistance and, at least initially, a relative deficiency of insulin secretion (Curtis *et al*, 2005). In absolute terms, the plasma insulin concentration (both fasting and meal-stimulated) usually is increased, although "relative" to the severity of insulin resistance, the plasma insulin concentration is insufficient to maintain normal glucose homeostasis (Ezenwaka*et al*, 2003; Freeman *etal*, 2013). With time, however, there is progressive beta cell failure and absolute insulin deficiency ensues. Recently, more sophisticated analyses of the β-cell response and regulation revealed that most subjects at risk for developing type 2 DM, that is those with combined impaired fasting glucose and impaired glucose tolerance already have a significant loss, close to 80% of the total insulin secretory capacity of the pancreas. The β cell membrane senses glucose and release glucokinase, inducing insulin secretion and transport of glucose across the cell membrane. In type 2, failure of effective release of glucokinase may be due to defects of β cell structure and function, receptor defects and availability of glucose.In a minority of type 2 diabetic individuals, severe insulinopenia is present at the time of diagnosis and insulin sensitivity is normal or near normal (Margullies*et al,* 2010). Although it may be genetic, the disorder is not associated with HLA autoantigens.

Most individuals with type 2 DM exhibit intra-abdominal (visceral) obesity, which is part of the “ectopic fat” deposition pattern closely related to the presence of insulin resistance. Insulin resistance is associated with obesity, hypertension, hyperlipidaemia and ischaemic heart disease (Curtis *et al*, 2005; Samreen*et al*, 2009). Onset is mostly during adult life (Crook *et al,* 2006; Afonja*et al*, 2011).

This type of diabetes often goes undiagnosed for many years and is associated with a strong genetic predisposition, with patients at increased risk with an increase in age, obesity, and lack of physical exercise. Characteristics usually include adult onset of the disease and milder symptoms than in type 1, with ketoacidosis rarely occurring. However, these patients are more likely to go into a hyperosmolar hyperglycaemic coma and are at an increased risk of developing macrovascular and microvascular complications.

2.2.1.3 Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) is defined as glucose intolerance, which is first recognized during pregnancy (Olarinoye*et al*, 2004). Causes of GDM include metabolic and hormonal changes. Patients with GDM frequently return to normal postpartum. In most women who develop GDM, the disorder has its onset in the third trimester of pregnancy. At least 6 weeks after the pregnancy ends, the woman should receive an oral glucose tolerance test and be reclassified as having DM, normal glucose tolerance, impaired glucose tolerance or impaired fasting glucose.

When the placenta develops, placental lactogen (hPL), which is diabetogenic as well as oestrogen, progesterone, prolactin, human chorionic gonadotrophins and cortisol are produced. Insulin resistance of pregnancy develops. If the beta cells cannot produce enough insulin to compensate for the insulin resistance, gestational diabetes develops in a susceptible mother (Afonja et al 2011).

Gestational diabetes mellitus complicates about 4% of all pregnancies worldwide. In the United Kingdom, it complicates about 4-5 % of pregnancies. It is associated with increased foetal abnormalities example high birth weights, cardiac defects and polyhydramnios. Other perinatal complications of GDM are respiratory distress syndrome, hypocalcaemia, neonatal hypoglycaemia and hyperbilirubinaemia (Mshelia*et al*, 2003). Also, maternal hypertension is commoner in women with GDM and the women are at increased

risk for development of diabetes in later years (Crook *et al*, 2006; Barbour *et al*,2010; Afonja*et al*, 2011;).

2.2.1.4 Other Specific Types of Diabetes

Genetic defects: Maturity Onset diabetes of the Young (MODY) is characterized by impaired insulin secretion with minimal or no insulin resistance. Patients typically exhibit mild hyperglycaemia at an early age. The disease is inherited in an autosomal dominant pattern and, at present, six different genetic abnormalities have been identified (Margullies*et al*, 2010). Genetic inability to convert proinsulin to insulin results in mild hyperglycaemia and is inherited as an autosomal dominant pattern. Similarly, the production of mutant insulin molecules has been identified in a few families and results in mild glucose intolerance. There are several genetic mutations that have been described in the insulin receptor and are associated with insulin resistance (Crook *et al*, 2006; Afonja*et al*, 2011).

Diseases of exocrine pancreas: Damage of the pancreas must be extensive for DM to occur. The most common causes are pancreatitis, trauma, and carcinoma. Cystic fibrosis and haemochromatosis also have been associated with impaired insulin secretion and DM(Crook *et al*, 2006; Margullies*et al*, 2010; Afonja*et al*, 2011).

Endocrinopathies: Since growth hormone, cortisol, glucagon, and epinephrine increase hepatic glucose production and induce insulin resistance in peripheral (muscle) tissues, excess production of these hormones can cause or exacerbate underlying DM. Although the primary mechanism of action of these counter regulatory hormones is the induction of insulin resistance in muscle and liver, overt type 2 DM does not develop in the absence of beta cell failure (Crook *et al*, 2006; Margullies*et al*, 2010; Afonja*etal*, 2011).

Infections: A variety of infections have been etiologically related to the development of DM. Of these, the most clearly established is congenital rubella. Approximately 20% of infants who are infected with the rubella virus at birth develop autoimmune type 2 DM later

in life. These individuals have the typical type 1 susceptibility genotype, DR3/DR4 (Crook *et al*, 2006; Margullies*et al,* 2010; Afonja*et al*, 2011).

2.2.2 Pathophysiology of Diabetes Mellitus

In both type 1 and type 2 diabetes, the individual will be hyperglycaemic, which can be severe. Glucosuria can also occur after the renal tubular transporter system for glucose becomes saturated. This happens when the plasma glucose concentration exceeds roughly 10 mmol/L in an individual with normal renal function and urine output. As hepatic glucose overproduction continues, the plasma glucose concentration reaches a plateau around 17–28 mmol/L). Provided renal output is maintained, glucose excretion will match the overproduction, causing the plateau. The patients of type 1 diabetes have a higher tendency to produce ketone bodies. Patients with type 2 diabetes seldom generate ketones but instead have a greater tendency to develop hyperosmolar non-ketotic hyperglycaemic states. The difference in glucagon and insulin concentrations in these two groups appears to be responsible for the generation of ketones through increased β-oxidation. In type 1, there is an absence of insulin with an excess of glucagon. This permits gluconeogenesis and lipolysis to occur. In type 2, insulin is present, as is (at times) hyperinsulinaemia; therefore, glucagon is attenuated. Fatty acid oxidation is inhibited in type 2. This causes fatty acids to be incorporated into triglycerides for release as very low density lipoproteins (VLDL). The laboratory findings of a patient with diabetes with ketoacidosis tend to reflect dehydration, electrolyte disturbances, and acidosis. Acetoacetate, β-hydroxybutyrate, and acetone are produced from the oxidation of fatty acids. The two former ketone bodies contribute to the acidosis. Lactate, fatty acids, and other organic acids can also contribute to a lesser degree. Bicarbonate and total carbon dioxide are usually decreased due to Kussmaul-Kien respiration (deep respirations). This is a compensatory mechanism to blow off carbon dioxide and

remove hydrogen ions in the process. The anion gap in this acidosis can exceed 16 mmol/L. Serum osmolality is high as a result of hyperglycaemia; sodium concentrations tend to be lower due in part to losses (polyuria) and in part to a shift of water from cells because of the hyperglycaemia. The sodium value should not be falsely underestimated because of hypertriglyceridaemia. Grossly elevated triglycerides will displace plasma volume and give the appearance of decreased electrolytes when flame photometry or prediluted, ion-specific electrodes are used for sodium determinations. Hyperkalaemia is almost always present as a result of the displacement of potassium from cells in acidosis. This is somewhat misleading because the patient's total body potassium is usually decreased. More typical of the untreated patient with type 2 diabetes is the nonketotic hyperosmolar state. The individual presenting with this syndrome has an overproduction of glucose; however, there appears to be an imbalance between production and elimination in urine. Often, this state is precipitated by heart disease, stroke, or pancreatitis. Glucose concentrations exceed 300 to 500 mg/dL (17–28 mmol/L) and severe dehydration is present. The severe dehydration contributes to the inability to excrete glucose in the urine. Mortality is high with this condition. Ketones are not observed because the severe hyperosmolar state inhibits the ability of glucagon to stimulate lipolysis. The laboratory findings of nonketotic hyperosmolar coma include plasma glucose values exceeding 55 mmol/L, normal or elevated plasma sodium and potassium, slightly decreased bicarbonate, elevated urea and creatinine, and an elevated osmolality (greater than 320 mOsm/dL). The gross elevation in glucose and osmolality, the elevation in BUN, and the absence of ketones distinguish this condition from diabetic ketoacidosis. Other forms of impaired glucose metabolism that do not meet the criteria for diabetes mellitus include impaired fasting glucose and impaired glucose tolerance (Bakari*et al*, 2004;Freeman *etal*, 2010).

2.2.3 Diagnosis of Diabetes Mellitus

Patients with diabetes mellitus present with polyuria, polydipsia, polyphagia and unexplained weight loss. The fasting blood glucose in adults is usually less than 5.1 mmol/L.

Diagnosis of diabetes mellitus is made when:

1. Fasting blood glucose is greater than or equal to 7.0 mmol/L on more than one occasion or greater than or equal to 6.1 mmol/L on more than one occasion among Africans.
2. Random blood glucose of more than or equal to 11.1 mmol/L with signs and symptoms of diabetes mellitus or greater than or equal to 10.0 mmol/L among Africans.
3. Two hour post glucose of more than or equal to 11.1 mmol/L.
4. Glycated haemoglobin of more than or equal to 6.5 % (AACE, 2012).

Any of the above criteria confirms the diagnosis of diabetes mellitus. If FBG is between 5.1 to 6.9 mmol/L on more than one occasion, oral glucose tolerance test should be carried out.

2.3 IMPAIRED GLUCOSE TOLERANCE

Impaired glucose tolerance (IGT) is diagnosed in those who have fasting blood glucose concentrations less than those required for a diagnosis of diabetes mellitus, but have a plasma glucose response during the OGTT between normal and diabetic states (Mshelia*et al*, 2003;Bakari*et al*, 2004;Olarinoye*et al*, 2004; Curtis *et al*, 2005). The 2 hour post load plasma glucose following an OGTT is 7.8 to 11.0 mmol/L for this classification. An OGTT is required to assign a patient to this class (Bakari*et al*, 2004). Development of overt diabetes occurs at a rate of 1 to 5% per year, but a large proportion of cases spontaneously revert to normal glucose tolerance. Microvascular disease is rare in this group, and patients usually do

not experience the renal or retinal complications of diabetes. Patients have an increased prevalence of atherosclerosis and mortality from cardiovascular disease.

2.4 IMPAIRED FASTING GLUCOSE

This category is analogous to IGT, but it is diagnosed by a *fasting* glucose value between those of normal and diabetic individuals, namely, fasting plasma glucose (FPG) between 5.6 and 6.9 mmol/L (Bakari*et al*, 2004;Curtis *et al*, 2005). It is a metabolic stage between normal glucose homeostasis and diabetes. As with IGT, persons with impaired fasting glucose (IFG) are at increased risk for the development of diabetes and cardiovascular disease. IFG and IGT are not clinical entities, but rather are risk factors for diabetes and cardiovascular disease (Bakari*et al*, 2004;Curtis *et al*, 2005).

2.5 HYPOGLYCAEMIA

By definition, hypoglycaemia is present if the plasma glucose concentration is less than 2.8 mmol/L in a specimen collected into a tube containing an inhibitor of glycolysis, for example fluoride oxalate. Blood cells continue to metabolize glucose in vitro, and low concentrations found in a specimen collected without such an inhibitor can be dangerously misleading (pseudohypoglycaemia).

2.5.1 Clinical Presentation

Symptoms of hypoglycaemia vary among individuals, and none is specific. Epinephrine produces the classic signs and symptoms of hypoglycaemia, namely, (1) trembling, (2) sweating, (3) nausea, (4) rapid pulse, (5) lightheadedness, (6) hunger, and (7) epigastric discomfort. These autonomic (neurogenic) symptoms are nonspecific and may be noted in other conditions, such as hyperthyroidism, pheochromocytoma, or even anxiety. Although controversial, it has been proposed that a rapid decrease in blood glucose may trigger the symptoms even though the blood glucose itself may not reach hypoglycaemic values, whereas gradual onset of hypoglycaemia may not produce symptoms.

The brain cannot store or produce glucose, and in resting adults the central nervous system (CNS) consumes approximately 50% of the glucose used by the body. Very low concentrations of plasma glucose < 2.5 mmol/L cause severe CNS dysfunction.

2.5.2 Causes of Hypoglycaemia

Causes of hypoglycaemia can be divided into:

a) Fasting hypoglycaemia: Occurs as a response to fasting. Some of the causes are insulinoma, non pancreatic tumours, glucagon deficiency, extra-pancreatic tumours, liver diseases, Addison's disease, hypopituitarism, malnutrition *etc.*

b) Reactive hypoglycaemia: this is stimulative i.e. it occurs due to some stimulous like hypoglycaemic drugs, alcohol, L-luecine, carbohydrate meal, vagotomy and galactosaemia.

c) Factitious hypoglycaemia: this condition may be induced by ingestion of large quantity of salicylates, monoamine oxidase inhibitors and barbiturates.

2.5.3 Investigations of Hypoglycaemia

It is very important to exclude pseudohypoglycaemia due to *in vitro* glucose metabolism, for example an old blood sample or one not collected into fluoride oxalate anticoagulant. Sometimes a cause may be evident from the medical and drug histories and clinical examination.

One of the most important tests in a patient with proven hypoglycaemia is to measure the plasma insulin and C-peptide concentrations when the plasma glucose concentration is low. Plasma for these assays should be separated from cells immediately and the plasma stored at –20°C until hypoglycaemia has been proven. These tests should differentiate exogenous insulin administration and endogenous insulin production, for example an insulinoma, from other causes of hypoglycaemia. Raised plasma insulin concentrations and suppressed plasma concentrations of C-peptide suggest exogenous insulin administration (hyperinsulinaemic hypoglycaemia). Conversely, a high plasma insulin and high C-peptide

can be seen in sulphonylurea or meglitinide administration, and a urine or plasma drug screen is thus important. Autoantibodies positive to the insulin receptor or insulin may also evoke hypoglycaemia. If a sulphonylurea drug screen and an insulin autoantibody screen are negative, raised plasma insulin and C-peptide concentrations are suggestive of an insulinoma.

2.6 HORMONES REGULATING BLOOD GLUCOSE CONCENTRATION

During a brief fast, a precipitous decline in the concentration of blood glucose is prevented by breakdown of glycogen stored in the liver and synthesis of glucose in the liver. Some glucose is derived from gluconeogenesis in the kidneys.These organs contain glucose-6-phosphatase, which is necessary to convert glucose-6-phosphate (derived from gluconeogenesis or glycogenolysis) to glucose. Skeletal muscle lacks this enzyme; muscle glycogen therefore cannot contribute directly to blood glucose. With more prolonged fasting (>42 hours), gluconeogenesis accounts for essentially all glucose production.In contrast, after a meal, the absorbed glucose is converted to glycogen or fat (for storage in the liver, skeletal muscle and adipose tissue). Despite large fluctuations in the supply and demand of carbohydrates, the concentration of glucose in the blood is maintained within a fairly narrow range by hormones that modulate the movement of glucose into and out of the circulation. These include insulin, which decreases blood glucose, and the counter-regulatory hormones (glucagon,epinephrine, cortisol, and growth hormone), which increase blood glucose concentrations. Normal glucose disposal depends on (1) the ability of the pancreas to secrete insulin, (2) the ability of insulin to promote uptake of glucose into peripheral tissue, and (3) the ability of insulin to suppress hepatic glucose production. The main insulin target organs are liver, skeletal muscle, and adipose tissue. These organs exhibit some differences in their responses to insulin. For example, the hormone stimulates glucose uptake through a specific glucose transporter—GLUT4—into muscle and fat cells, but not into liver cells (sacks *et al*, 2006; Crook *et al*, 2006; Pittman *et al*, 2009, Freeman *et al*, 2010).

2.6.1 Insulin

Insulin is a protein hormone produced by the β-cells of the islets of Langerhans in the pancreas. Insulin was the first protein hormone to be sequenced, the first substance to be measured by radioimmunoassay (RIA), and the first compound produced by recombinant DNA technology for clinical use (Igharo*et al.*,2009). It is an anabolic hormone that stimulates the uptake of glucose into fat and muscle, promotes the conversion of glucose to glycogen or fat for storage, inhibits glucose production by the liver, stimulates protein synthesis, and inhibits protein breakdown (Pittman *et al*, 2009).

2.6.1.1Chemistry

Human insulin [molecular weight (MW) 5808 Da] consists of 51 amino acids in two chains (A and B) joined by two disulfide bridges, with a third disulfide bridge within the A chain. The amino acid sequence of human insulin differs slightly from insulin of other species. The most commonly used forms now are recombinant human insulins (sacks *et al*, 2006; Crook *etal*, 2006; Pittman *et al*, 2009, Freeman *et al*, 2010).

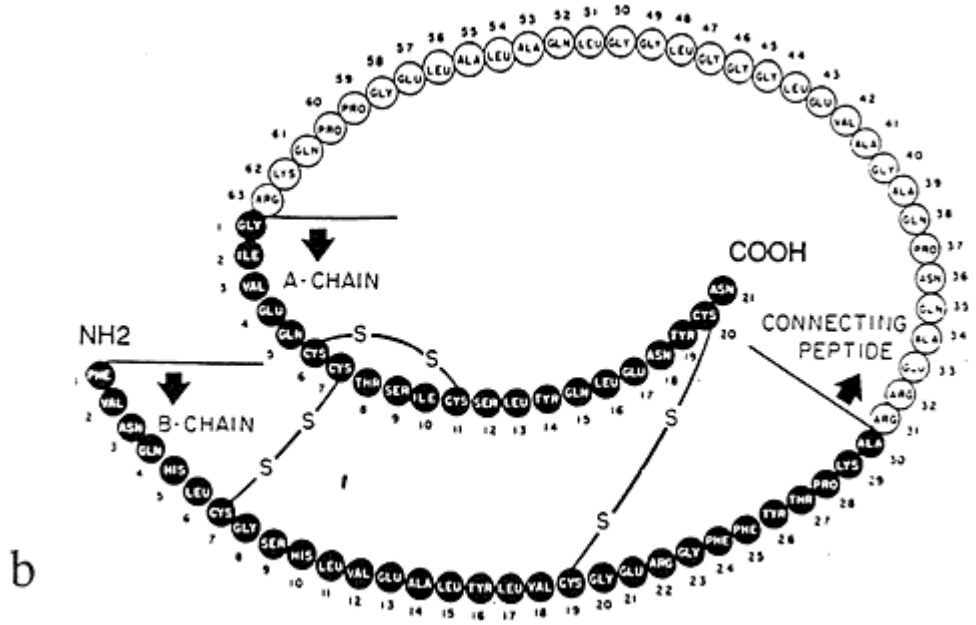


Figure 2.1: Primary structure of porcine insulin. The sequence of human insulin is identical to that of porcine insulin except for the change of AlaB30 to ThrB30 in human insulin.

2.6.1.2 Synthesis

Preproinsulin, a protein of about 100 amino acids (MW 12,000 Da), is formed by ribosomes in the rough endoplasmic reticulum of the pancreatic β-cells. Preproinsulin is not detectable in the circulation under normal conditions because it is rapidly converted by cleaving enzymes to proinsulin (MW 9000 Da), an 86 amino acid polypeptide. This is stored in secretory granules in the Golgi complex of the β-cells.Cleavage of proinsulin is catalyzed by two Ca2+-regulated endopeptidases: prohormoneconvertases 1 and 2 (PC1 and PC2).PC1 (sometimes designated PC3) hydrolyzes the molecule on the *C*-terminal end of Arg-31 and Arg-32 (at the BC junction) to yield split-32, 33-proinsulin. PC2 cleaves proinsulin on the *C*-terminal side of dibasic residues Lys-64 and Arg-65 (at the AC junction) to generate split-65, 66-proinsulin. Each enzymatic hydrolysis is rapidly followed by the removal of two newly exposed *C*-terminal basic amino acids by carboxypeptidase-H to produce insulin and C-peptide (Sacks *et al*, 2006; Crook *et al*, 2006; Pittman *et al*, 2009, Freeman *et al*, 2010).

2.6.1.3 Release

Glucose, amino acids, pancreatic and gastrointestinal hormones (e.g., glucagon, gastrin, secretin, pancreozymin, gastrointestinal polypeptide), and some medications (e.g.,sulfonylureas, β-adrenergic agonists) stimulate insulin secretion (Inzucchi*et al*, 2012) . Insulin release is inhibited by hypoglycaemia, somatostatin (produced in the pancreatic δ-cells), and various drugs (e.g., α-adrenergic agonists, β-adrenergic blockers, diazoxide, phenytoin, phenothiazines, nicotinic acid).In healthy individuals, insulin is secreted in a pulsatile fashion, with glucose and insulin the main signals in the feedback loop.Glucose elicits the release of insulin from the pancreas in two phases. The first phase begins soon after intravenous injection of glucose and ends within 10 minutes. The second phase, beginning at the point where the first phase ends, depends on continuing insulin synthesis and release and lasts until normoglycaemia has been restored, usually within one to two hours. With

progressive failure of β-cell function, the first phase insulin response to glucose is lost, but other stimuli such as glucagon or amino acids may be able to elicit this response. Although the second-phase insulin response is preserved in most patients with type 2 diabetes mellitus, both the first-phase response and normal pulsatile insulin secretionare lost. In contrast, patients with type 1 diabetes mellitus produce minimal or no insulin response (Pittman *et al*, 2009; Freeman *et al*, 2010; Afonja*et al*, 2011).

2.6.1.4 Degradation

On the first pass through the portal circulation, approximately 50% of insulin is extracted by the liver, where it is degraded. Because the amount extracted is variable, plasma insulin concentrations may not accurately reflect the rate of insulin secretion. Additional insulin degradation occurs in the kidneys. Insulin is filtered through the glomeruli, reabsorbed,and degraded in the proximal tubule. The basal insulin secretory rate is about 1 U (43 μg)/h, with total daily secretion of about 40 U. The half-life of insulin in the circulation is between 4 and 5 minutes (Pittman et al, 2009).

2.6.2 Glucagon

Glucagon is a 29 amino acid polypeptide secreted by α-cells of the pancreas (Afonja*et al*, 2011). The major target organ for glucagon is the liver, where it binds to specific receptors and increases both intracellular adenosine-5′-monophosphate and calcium. Glucagon stimulates the production of glucose in the liver by glycogenolysis and gluconeogenesis (Afonja*et al*, 2011).In addition, glucagon enhances ketogenesis in the liver. A minor target organ for glucagon is adipose tissue, where it increases lipolysis. Glucagon secretion is regulated primarily by plasma glucose concentrations, with hypoglycaemia and hyperglycaemia being stimulatory and inhibitory respectively (Afonja*et al*, 2011). Long-standing diabetes mellitus impairs the glucagon response to hypoglycemia, resulting in an increased incidence of hypoglycaemic episodes. Stress, exercise, and amino acids induce

glucagon release. Insulin inhibits glucagon release from the pancreas and decreases glucagon gene expression, thereby attenuating its biosynthesis. Increased glucagon concentrations, secondary to insulin deficiency, are believed to contribute to the hyperglycaemia and ketosis of diabetes (Sacks *et al*, 2006; Crook *et al,* 2006; Pittman *et al*, 2009, Freeman *et al*, 2010).

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2.6.3 Epinephrine

Epinephrine, a catecholamine secreted by the adrenal medulla, stimulates glucose production (glycogenolysis) and decreases glucose use, thereby increasing blood glucose concentrations. It also stimulates glucagon secretion and inhibits insulin secretion by the pancreas. Epinephrine appears to have *a key role in glucose counter-regulation when glucagon secretion is impaired* (e.g., in type 1 diabetes mellitus). Physical or emotional stress increases epinephrine production, releasing glucose for energy. Tumors of the adrenal
medulla, known as pheochromocytomas,secrete excess epinephrine or norepinephrine and produce moderate hyperglycemia as long as glycogen stores are available in the liver (Sacks *et al*, 2006; Crook *et al*, 2006; Pittman *et al*, 2009, Freeman *et al*, 2010).

2.6.4 Growth Hormone

Growth hormone is a polypeptide secreted by the anterior pituitary gland. It stimulates gluconeogenesis, enhances lipolysis, and antagonizes insulin-stimulated glucose uptake (Frier*et al*, 1999).

2.6.5 Cortisol

Cortisol, secreted by the adrenal cortex in response to adrenocorticotropic hormone (ACTH), stimulates gluconeogenesis and increases the breakdown of protein and fat. Patients with Cushing's syndrome have increased cortisolowing to tumour or hyperplasia of the adrenal cortex and may become hyperglycaemic. In contrast, people with Addison's disease have

adrenocortical insufficiencycaused by destruction or atrophy of the adrenal cortex and may exhibit hypoglycaemia (Frier*et al*, 1999).

2.7 MARKERS OF GLYCAEMIC CONTROL

Biological markers used in the assessment of glycaemic control in diabetic patients include plasma glucose, glycated haemoglobin (HbA1c) and fructosamine.

2.7.1 Glucose

Plasma glucose concentration either random or fasting specimens are commonly used in Africa for diagnosis and follow up of diabetic patients. Blood for plasma glucose estimation should be taken if a patient presents with symptoms of diabetes mellitus or glycosuria or if it is desirable to exclude the diagnosis, for example because of a strong family history.

Blood samples may be taken:

A) After 10 - 12 hours fasting,

B) At random,

C) As part of an oral glucose load test.

Diabetes mellitus is confirmed if one of the following is present:

a fasting venous plasma concentration of 7.0 mmol/L or more on two occasions or once with symptoms or a random venous plasma concentration of 11.1 mmol/L or more on two occasions or once with symptoms.

Diabetes mellitus is unlikely if the fasting venous plasma glucose concentration is less than 5.5 mmol/L on two occasions. Samples taken at random times after meals are less reliable for excluding than for confirming the diagnosis.

The indications for performing an Oral Glucose Tolerance Test (OGTT) to diagnose diabetes mellitus may include:

A) Impaired fasting glucose with fasting venous plasma glucose concentration between 6.1 mmol/L and less than 7.0 mmol/L (Mshelia*et al* 2003; Bakari*et al*, 2004;Curtis *et al*, 2005).

B) Impaired glucose tolerance with plasma glucose concentration between 7.8 mmol/L to 11.1 mmol/L (Mshelia*et al* 2003; Bakari*et al*, 2004;Curtis *et al*, 2005).

C) Potential diabetics, subjects who are first degree relatives of diabetic patients and are 25 years of age or older (Mshelia*et al* 2003).

D) A second twin of type 2 diabetic patient (Mshelia*et al* 2003).

E) Diagnosis of gestational diabetes mellitus (Mshelia*et al* 2003;Olarinoye*et al*, 2004).

F) Useful in the diagnosis of acromegaly, glucagonoma, pheochromocytomaetc (Mshelia*et al* 2003).

G) Unexplained retinopathy or nephropathy in an individual with normal fasting plasma glucose.

H) Poor obstetric history like frequent miscarriages, congenital anomaly, polyhydramnious, macrosomia, stillbirth and intrauterine foetal death (Mshelia*et al* 2003).

2.7.2 Glycated Haemoglobin

Glycation is the non-enzymatic and irreversible addition of a sugar residue to amino groups of proteins (Edo *et al*, 2012).Human adult haemoglobin (Hb) usually consists of HbA (97% of the total), HbA2 (2.5%), and HbF (0.5%). HbA is made up of four polypeptide chains, two α- and two β-chains. Chromatographic analysis of HbA identifies several minor haemoglobins, namely, HbA1a, HbA1b, and HbA1c, which are collectively referred to as HbA1, fast haemoglobins. HbA1c is formed by the condensation of glucose with the *N*-terminal valine residue of each β-chain of HbA to form an unstable Schiff base. The Schiff

base may dissociate or may undergo an Amadori rearrangement to form a stable ketoamine, HbA1c. HbA1a1 and HbA1a2, which make up HbA1a, have fructose-1,6- diphosphate and glucose-6-phosphate, respectively, attached to the amino terminal of the β-chain. The structure of HbA1b, identified by mass spectrometry, contains pyruvic acid linked to the amino terminal valine of the β- chain, probably by a ketamine or enamine bond. HbA1c is the major fraction,constituting approximately 80% of HbA1. Formation of GHb is essentially irreversible, and the concentration in the blood depends on both the lifespan of the red blood cell (RBC; average lifespan is 120 days) and the blood glucose concentration. Because the rate of formation of GHb is directly proportional to the concentration of glucose in the blood, the GHb concentration represents integrated values for glucose over the preceding 8 to 12 weeks (Edo *et al*, 2010).This provides an additional criterion for assessing glucose control because GHb values are free of day-to-day glucose fluctuations and are unaffected by recent exercise or food ingestion (AACE, 2012).

2.7.3 Fructosamine

2.7.3.1 Introduction

Fructosamines are compounds formed by a nonenzymatic reaction between glucose and an amino group on a protein. Fructosamine testing enables assessment of long-term glycaemic control usually two to three weeks.

The reference range of fructosamine is 200-285 mmol/L (Burris *et al*, 2006).

2.7.3.2 Clinical Significance

In selected patients with diabetes mellitus (e.g., gestational diabetes mellitus, change in therapy), assays may be needed that are more sensitive than HbA1c to shorter-term alterations in average blood glucose concentrations. Nonenzymatic attachment of glucose to amino groups of proteins other than haemoglobin (e.g., serum proteins, membrane proteins, lens crystallins) to form ketoamines also occurs. Because serum proteins turn over more

rapidly than erythrocytes (the circulating half-life for albumin is about 20 days), the concentration of glycated serum albumin reflects glucose control over a period of 2 to 3 weeks. Therefore, both deterioration of control and improvement with therapy are evident earlier than with GHb. Fructosamineis the generic name for plasma protein ketoamines. The name refers to the structure of the ketoamine rearrangement product formed by the interaction of glucose with the ε-amino group on lysine residues of albumin. Analogous to GHb, measurement of fructosamine may be used as an index of the average concentration of blood glucose over an extended period of time (Isa,1990) but one that is about as long as the time examined with GHb. Because all glycated serum proteins are fructosamines and albumin is the most abundant serum protein, measurement of fructosamine is thought to be largely a measure of glycated albumin, but this has been questioned by some investigators.

Because fructosamine determination monitors short-term glycaemic changes different from GHb, it may have a role in conjunction with GHb rather than instead of it. In addition, fructosamine may be useful in patients with haemoglobin variants, such as HbS or HbC, that are associated with decreased erythrocyte lifespan, where GHb is of little value. Gross changes in protein concentration and half-life may have large effects on the proportion of protein that is glycated. Thus fructosamine results may be invalid in patients with nephrotic syndrome, cirrhosis of the liver, or dysproteinaemias, or after rapid changes in acute-phase reactants.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY LOCATION

This study was conducted in the departments of Medicine and Chemical Pathology of Ahmadu Bello University Teaching Hospital (ABUTH) Zaria.Zaria is a major city in Kaduna state in Northern Nigeria. It comprises two local government areas; namely, Zaria and Sabon-Gari Local Government Areas. The residents of Zaria City are predominantly Hausa-Muslims, while Sabon-Gari is inhabited predominantly by southern migrants who are mainly Christians. Zaria is situated on the large extended undulating plains of Northern Nigeria, extending almost unbroken from Sokoto to Lake Chad and beyond. Zaria is at a height of about 652.6 meters above sea level and 950 km from the coast. It is in the centre of Northern Nigeria with a latitudinal position of 110° 31′ North and 70° 42′East. It is bounded to the North by Makarfi, to the south by Igabi, to the east by Soba and to the west by Giwa Local Government Areas of Kaduna State. The two local government areas constituting the study area, Zaria and Sabon-Gari Local Governments have 408,198 and 286,871 population respectively. Thus, bringing the total population of the study area to 695,069 people (Inomebe*et al*, 2009).

3.2 STUDY POPULATION

Eighty eight diabetic patients attending various diabetic clinics in ABUTH were recruited into the study, while 88 apparently healthy non diabetic subjects were recruited as controls.

3.2.1 Inclusion criteria

All consenting adult type 2 diabetic patients not on any form of insulin treatment attending ABU Teaching Hospital, Zaria and apparently healthy controls.

3.2.2 Exclusion criteria

Patients with type 1 diabetes mellitus, patients on insulin treatment and those who decline to consent to the study were excluded from the study.

3.2.3 Sample size determination

Estimated sample size was calculated using the formula below (Singha, 1996).

$n= z^2pq/d^2$,

Where n=sample size, z= confidence interval (1.96), d= precision (0.05),

P=prevalence in target population with characteristic being measured in Nigeria in 2013 (4.99 %) (IDF; 2013), q=1-p

n = 73

Attrition rate of 20 % is considered = 17

Total sample size = 73 + 15

Total sample size = 88

A sample size of eighty eight 88 diabetic patients were recruited into the study and 88 apparently healthy non diabetic individuals were considered as controls.

3.2.4 Ethical approval

Ethical approval was obtained from Ethical Committee of Ahmadu Bello University Teaching Hospital, Zaria in accordance with Helsinki declaration (*Appendix I*).

3.2.5 Informed consent

Informed written consents were obtained from all the participants in the study after a detailed nature of the study was explained to each of them (*Appendix II*).

3.2.6 Sampling

Clinical information was obtained from the diabetic patients and controls using a preformed questionnaire (A*ppendix III)*.

3.3 SAMPLE COLLECTION AND PROCESSING

Seven millilitres of fasting blood specimens was collected into plain tubes by venepuncture of an antecubital vein using syringe and needle after sterilizing the antecubital fossa with methylated spirit. Five millilitres was allowed to clot within 30 minutes and centrifuged at 4000 rpm for 10 minutes to separate the serum from the cells. The sera were carefully drawn into sample bottles and then frozen at -20 ºC. This was used for the analysis of glucose, fructosamine and insulin. The remaining two millilitres of blood were collected into EDTA bottle by venepuncture of an antecubital vein using syringe and needle after sterilizing the site with methylated spirit for analysis of HbA1c assay.

3.4 LABORATORY ANALYTICAL METHODS

3.4.1 Serum Insulin Concentration (ELISA, Andersen *et al;* 1993).

The insulin kit was acquired from MonobindInc, USA. Its sensitivity was 0.182 μIU/ml, within run and between run coefficient of variation were less than 8.3 and 11.3 % respectively.

3.4.1.1 Principle

The wells were coated with monoclonal antibody with higher activity for insulin. When the samples and controls were incubated in the wells with the enzyme conjugate, a sandwich complex bound to the wells is formed. Unbound conjugates were then washed off with the buffer. The amount of bounded peroxidase was proportional to the concentration of insulin present in the sample. Upon addition of the substrate and chromogen, the intensity of colour was developed in proportion to the insulin concentration in the samples.

3.4.1.2 Procedure

Twenty five microlitres (25 μL) of serum sample, controls and calibrators were dispensed into assigned wells, and then 100 μL of enzyme conjugate were added into each well and mixed for 5 seconds. This was followed by incubation for 30 minutes at 25 °C. Washing was done with 300 μL wash buffer five times and 100 μL of tetramethylbenzidine (TMB) solution was dispensed into each well and then incubated for 15 minutes at room temperature. The reaction was stopped by adding 50 μL of stop solution (2N HCL) to each well and absorbance was read at 450 nm wavelength within 5 minutes.

3.4.1.3 Calculation of results: The results (μIU/ml) were obtained from the calibration curve, see appendix IV.

3.4.2 Glycated Haemoglobin (HbA1c) (Ion exchange low pressure liquid chromatography, Eckfeldt*et al*; 1997).

The HbA1c kit was acquired from ALLPRO Inc, China and used on Audicom Automated analyzer. The kits sensitivity was 0.1 %, within run and between run coefficient of variations were less than 0.3 %.

3.4.2.1 Principle

The cation exchange resin (negatively), packed in disposable minicolumn, has an affinity for haemoglobin, which is positively charged. The positive ion exchange resin is treated with buffer to make it have negative charge and thus producing affinity with Hb positive charge. Since $HbA1_{a,}$ $HbA1_b$, HbA1c and HbA_0 have different position charges, they have different adhesion to the resin.

1. A- eluent with pH 6.7 and having the lowest ion strength mainly make the resin recover the lowest ion strength and plays the role of flushing and balancing the resin.
2. B- eluent with pH 6.7 and having the lower ion strength elutriates $HbA1_a$ and$HbA1_b$ with less positive charge and weaker adhesion.
3. C- eluent with pH 6.4 and having the higher ion strength elutriates $HbA1_c$ with more positive charge and stronger positive adhesion.
4. B- eluent with pH 6.7 and having the highest ion strength elutriates $HbA1_0$ with the strongest positive charge and plays role of regenerating resin.

With the method of single wavelength (415 nm), the absorbance of eluate is continuously measured on line to obtain the corresponding chromatogram of haemoglobin. The integral area of $HbA1_{ab,}$ $HbA1_c$ and HbA_0 absorbance can be calculated with integral principle.

3.4.2.2 Procedure

Ten microliter of whole blood from EDTA bottle was titrated in to an eppendop tube containing 300 µL of haemolytic reagent and incubated at 50 °C for 5 minutes before transfer

into sample tray of the auto-analyzer which automatically produces results after pressing command botton.

3.4.2.3 Calculation of results: The results (%) were obtained from an inbuilt calibration curve.

3.4.3 Serum Fructosamine Concentration:

Using Nitro Blue Tetrazonium (NBT) spectrophotometric analysis (Johnson *et al,* 1983). The kit was acquired from ALLPRO Inc. China. Its sensitivity was 0.1 µmol/L and both within run and between run coefficient of variations were less than 10 %.

3.4.3.1 Principle

Fructosamine in an alkaline medium reduces certain tetrazonium salts, such as Nitro Blue Tetrazonium (NBT) to produce a reddish brown colour which is read spectrophotometrically at 520 nm wavelength. The intensity of colour produced is proportional to the concentration of fructosamine in the sample.

3.4.3.2 Procedure

To 1.0 ml of NBT reagent in carbonate buffer, one hundred microlitre of sample was added. It was then mixed thoroughly and incubated at 37°C. Absorbance was measured at 520 nm wavelength. Distilled water was used as blank.

3.4.3.3 Calculation

Concentration of serum fructosamine = Absorbance of test/Absorbance of standard x concentration of standard (µmol/L)

3.4.4 Serum Glucose:

Using glucose oxidase spectrophotometric method (Trinder, 1969). The kit was acquired from ALLPRO Inc, China. Its sensitivity was 1 mg/dl while it's within run and between run coefficient of variations were less than 8 % and 11 % respectively.

3.4.4.1 Principle

Glucose oxidase is a specific enzyme which catalyzes oxidation of aldehyde group of glucose to gluconic acid with production of equivalent amount of hydrogen peroxide. The peroxide formed is broken down to water and oxygen by peroxidase. In the presence of phenol, the oxygen from peroxide is transferred to a suitable oxygen acceptor (4- amino phenazone) with production of a red coloured end product which is measured spectrophotometrically at 510 nm. The intensity of the colour formed is directly proportional to the concentration of glucose in the blood.

3.4.4.2 Procedure

Three test tubes were prepared and labelled as blank, test and standard. Phenol reagent (4 ml) was pipetted into each tube. Glucose colour reagent (1 ml) was also be added into each tube. Distilled water, serum and standard were added into the tubes labelled blank, test and standard respectively. The contents were well mixed and incubated at 37 ℃ for 20 minutes and absorbance was taken at 510 nm using blank to set the spectrophotometer to zero.

3.4.4.3 Calculation

Concentration of serum glucose = Absorbance of test/Absorbance of standard x concentration of standard (mmol/L).

3.5 DATA ANALYSIS

The data were analyzed using EPI-Info 3.5.3 statistical software and micro excel 2007 version. The mean values of blood pressure, age, body mass index, blood glucose, HbA1c, fructosamine and insulin were compared in patients and controls and p-values obtained using kruskal Wallis test (Equivalent to Chi-square) of difference between two means. Mean values of insulin was also correlated with body mass index after log transformation. The data were exported to Microsoft excel for graph and line of best fit determining the relationship between insulin and blood glucose, fructosamine, HbA1c and body mass index among the diabetic patients. Regression equations were also obtained from Microsoft excel. Homa calculator version 2.2.3 was used to calculate insulin resistance from Homeostatic Model Assessment of Insulin resistance (HOMA – IR) was derived from software downloaded from http://www.dtu.ox.ac.uk/homa. p - values less than 0.05 was considered statistically significant.

3.6 QUALITY CONTROL

Adequate quality control was observed when carrying out the analysis of the samples to ensure that the results obtained are reliable. This was done by analysing the samples in a batch together with quality control sera for specific analytes. The quality control sera (medium and high) are commercially prepared from Allpro Corporation (The manufacturers of the kits used).

CHAPTER FOUR

4.0 RESULTS

4.1 CLINICAL CHARACTERISTICS OF THE STUDY POPULATION

The clinical characteristics of the study population are shown in table 4.1. Eighty eight diabetic patients and 88 non diabetic healthy controls were recruited into the study. Ages (mean ± S. D) were 45.41 ± 15.98 and 42 ± 14.83 years among diabetic patients and healthy controls respectively (p = 0.0621). The mean ± SD of systolic blood pressure (132.73 ± 18.48 mmHg) was significantly higher among diabetic patients than the value in healthy non-diabetic controls 122.31 ± 22.80 mmHg (p = 0.0006). There was no significant difference in the mean diastolic blood pressure between the diabetic subjects (mean ± SD) 82.78 ± 10.80 mmHg and non-diabetic controls (mean ± SD) 81.10 ± 13.33 mmHg (p=0.1979). The mean value of body mass index in diabetic subjects was higher than the non-diabetic healthy controls (mean ± SD) 23.25 ± 6.05 Kg/m^2 and 27.12 ± 6.17 Kg/m^2 respectively (p=0.0000).

4.2 BIOCHEMICAL CHARACTERISTICS OF THE CONTROLS AND DIABETIC SUBJECTS

The biochemical characteristics of non diabetic healthy controls and diabetic patients are shown in table 4.2. Insulin, insulin resistance, FBG, HbA1c and fructosamine were significantly higher in the diabetic subjects than in the non-diabetic healthy controls (p=0.0000).

Table 4.1: Clinical characteristics of controls and diabetic patients (Mean ± SD).

Subjects	n	Age (years)	SBP (mmHg)	DBP (mmHg)	BMI (Kg/m^2)
Diabetics	88	45.41±15.98	132.73 ± 18.48	82.78 ± 10.80	27.12 ± 6.17
Controls	88	42.36±14.83	122.31 ± 22.80	81.10 ± 13.33	23.25 ± 6.05
p-value		0.0621	0.0006	0.1979	0.0000

SBP = Systolic Blood Pressure

DBP = Diastolic Blood Pressure

BMI = Body Mass Index

Table 4.2: Biochemical characteristics of non-diabetic controls and diabetic patients (mean ± SD)

Subjects	n	FBG (mmol/L)	Insulin (µIU/ml)	Fructosamine (µmol/L)	HbA1c (%)	IR
Diabetics	88	9.70 ± 4.56	12.98 ± 8.90	467.56 ± 225.27	9.87 ± 3.49	3.51 ± 10.34
Controls	88	4.32 ± 0.76	5.23±2.65	239.10 ± 30.34	4.61±1.07	1.53 ± 1.21
p – value		0.0000	0.0000	0.0000	0.0000	0.0231

FBG = Fasting Blood Glucose

HbA1c = GlycatedHaemoglobin

4.3RELATIONSHIP BETWEEN SERUM FASTING INSULIN (log transformed), INSULIN RESISTANCE, FBG, HBA1C, FRUCTOSAMINE AND BODY MASS INDEX (BMI) AMONG NON-DIABETIC CONTROLS

Relationship between serum log transformed Fasting Insulin concencentration, FBG, HbA1c, fructosamine and insulin resistance with Body Mass Index (BMI) in non-diabetic controls is shown in table 4.3. There was no significant relationship between BMI, insulin, insulin resistance and markers of glycaemic control ($p>0.05$).

4.4RELATIONSHIP BETWEEN SERUM FASTING INSULIN (log transformed), INSULIN RESISTANCE, FBG, HBA1C, FRUCTOSAMINE AND BODY MASS INDEX (BMI) AMONG DIABETIC PATIENTS

Relationship between serum log transformed Fasting Insulin concentration, FBG, HbA1c, fructosamine levels and Body Mass Index (BMI) among diabetic patients is shown in table 4.4. There was significant and steady increase in fasting serum insulin concentration with increase in body mass index among diabetic patients ($p=0.0291$). Other markers of glycaemic control i.e. HbA1c, fasting blood glucose and fructosamine did not show specific pattern and were not statistically significant ($p>0.05$). Insulin resistance was lowest among those with normal BMI and was significantly higher in obese than in those with low and normal BMI.

Table 4.3: Relationship between serum Fasting Insulin concentration (log transformed), insulin resistance, FBG, HbA1c, fructosamine (mean ± SD) and Body Mass Index (BMI) in non-diabetic healthy controls

Subjects	n	Insulin (µIU/ml)	FBG (mmol/L)	HbA1c (%)	Fructosamine (µmol/L)	IR
Under weight	16	4.15 ± 1.58	4.03 ± 0.82	3.99 ± 0.52	242.49 ± 47.56	1.58 ± 0.37
Normal BMI	46	5.25 ± 1.56	4.50 ± 0.76	4.66 ± 1.10	237.68 ± 25.36	1.41 ± 0.57
Overweight	18	4.44 ± 1.42	4.22 ± 0.72	4.92 ± 1.24	238.34 ± 36.42	1.90 ± 0.75
Obese	08	3.24 ± 1.08	3.74 ± 0.12	4.80 ± 1.01	244.73 ± 14.74	1.18 ± 0.53
p-value		0.1341	0.1180	0.1194	0.9510	0.1198

BMI = Body Mass Index

FBG = Fasting Blood Glucose

HbA1c = GlycatedHaemoglobin

IR = Insulin resistance

Classification of BMI (Kg/m^2)

BMI < 18.6 = Underweight

BMI 18.6 – 24.9 =Normal

BMI 25.0 – 29.9 =Overweight

BMI Above 30 =Obese

Table 4.4: Relationship between serum Fasting Insulin, insulin resistance (log transformed), FBG, HbA1c, fructosamine levels (mean ± SD) and Body Mass Index (BMI) among diabetic patients

Subjects	n	Insulin (µIU/ml)	FBG (mmol/L)	HbA1c (%)	Fructosamine (µmol/L)	IR
Under weight	6	8.80 ± 1.50[a]	14.05 ± 5.51	13.03 ± 5.60	646.28 ± 243.68	3.87 ± 0.79
Normal BMI	29	9.69 ± 1.98[b]	9.21 ± 4.56	9.73 ± 3.66	477.03 ± 253.80	3.79 ± 1.61
Overweight	27	10.84 ± 1.68	9.94 ± 4.90	9.93 ± 3.20	455.34 ± 209.46	4.39 ± 2.46
Obese	26	14.21 ± 1.84	9.78 ± 4.02	9.88 ± 3.19	468.18 ± 206.76	7.05 ± 18.56[c]
p-value		0.0291	0.3421	0.5833	0.6482	0.0350

a = statistically significant difference between underweight and obese

b = statistically significant difference between normal BMI and obese

c = statistically significant difference between normal BMI, over weight and obese

BMI = Body Mass Index

FBG = Fasting Blood Glucose

HbA1c = GlycatedHaemoglobin

IR = Insulin resistance

Classification of BMI (Kg/m^2)

BMI < 18.6 = Underweight

BMI 18.6 – 24.9 =Normal

BMI 25.0 – 29.9 =Overweight

BMI above 30 =Obese

4.5: FASTING INSULIN LEVELS (MEAN ± SD) IN DIABETIC PATIENTS WITH GOOD AND POOR GLYCAEMIC CONTROL USING FBG AS A MARKER.

Mean values of insulin levels in diabetic patients with good and poor glycaemic control using FBG as a marker are shown in table 4.8. The mean values of Insulin in diabetic patients with good glycaemic control was significantly lower than those with poor glycaemic control.

4.6: FASTING INSULIN LEVELS (MEAN ± SD) IN DIABETIC PATIENTS WITH GOOD AND POOR GLYCAEMIC CONTROL USING HBA1C AS A MARKER.

Insulin levels (mean ± SD) in diabetic patients with good and poor glycaemic control using HbA1c as a marker are shown in table 4.9. The mean values of Insulin in diabetic patients with good glycaemic control was significantly lower than those with poor glycaemic control.

4.7: FASTING INSULIN LEVELS (MEAN ± SD) IN DIABETIC PATIENTS WITH GOOD AND POOR GLYCAEMIC CONTROL USING FRUCTOSAMINE AS A MARKER.

The mean ± SD values of insulin in diabetic patients with good and poor glycaemic control using fructosamine as a marker are shown in table 4.10. The mean values of Insulin in diabetic patients with good glycaemic control was significantly lower than those with poor glycaemic control.

Table 4.5: Fasting insulin levels (mean ± SD) in diabetic patients with good and poor glycaemic control using FBG as a marker

Glycaemic control	n	Insulin µIU/ml
Good glycaemic control	35	7.85 ± 3.35
Poor glycaemic control	53	16.31 ± 9.84
p-value		0.0000

Good glycaemic control indicated by FBG < 7.0 mmol/L

Poor glycaemic control indicated by FBG ≥7.0 mmol/L

n = frequency

SD = Standard deviation

Table 4.6: Fasting insulin levels (mean ± SD) in diabetic patients with good and poor glycaemic control using HbA1c as a marker.

Glycaemic control	n	Insulin µIU/ml
Good glycaemic control	23	7.05 ± 2.88
Poor glycaemic control	65	15.08 ± 9.37
p-value		0.0000

Good glycaemic control indicated by HbA1c < 7.0 %

Poor glycaemic control indicated by HbA1c ≥ 7.0 %

n = frequency

SD = Standard deviation

Table 4.7: Fasting insulin levels (mean ± SD) in diabetic patients with good and poor glycaemic control using fructosamine as a marker.

Glycaemic control	n	Insulin µmol/L
Good glycaemic control	20	7.03 ± 3.10
Poor glycaemic control	68	14.76 ± 9.28
p-value		0.0001

Good glycaemic control indicated by fructosamine< 286 µmol/L

Poor glycaemic control indicated by fructosamine≥286 µmol/L

n = frequency

SD = Standard deviation

4.8 RELATIONSHIP BETWEEN FASTING INSULIN LEVELS WITH FASTING BLOOD GLUCOSE, HbA1c AND FRUCTOSAMINE IN DIABETIC PATIENTS

The relationship between fasting insulin and fasting blood glucose in diabetic patients is shown in Figure 4.1. Fasting insulin concentrations increase with increase in fasting blood glucose (r^2=0.5265, p = 0.0000).

The relationship between fasting insulin concentrations and HbA1c concentrations (%) in diabetic patients is shown in Figure 4.2. Fasting insulin concentrations increase with increase in HbA1c (r^2=0.4699, p = 0.0000).

The relationship between fasting insulin concentrations and fructosamine concentrations in diabetic patients is shown in Figure 4.3. Fasting insulin concentrations increase with increase in fructosamine concentration (r^2=0.4729, p = 0.0001).

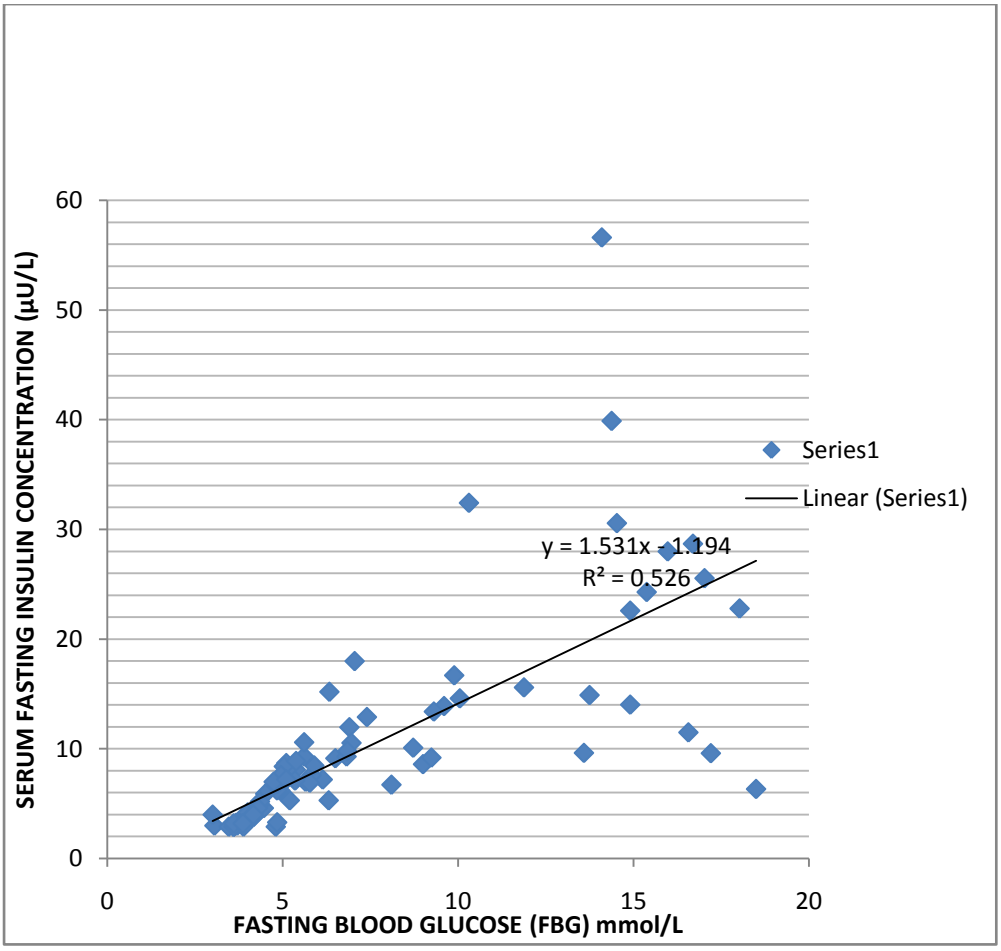


Figure 4.1: Relationship between fasting insulin and blood glucose levels in diabetic patients (r = +0.73, p = 0.0000)

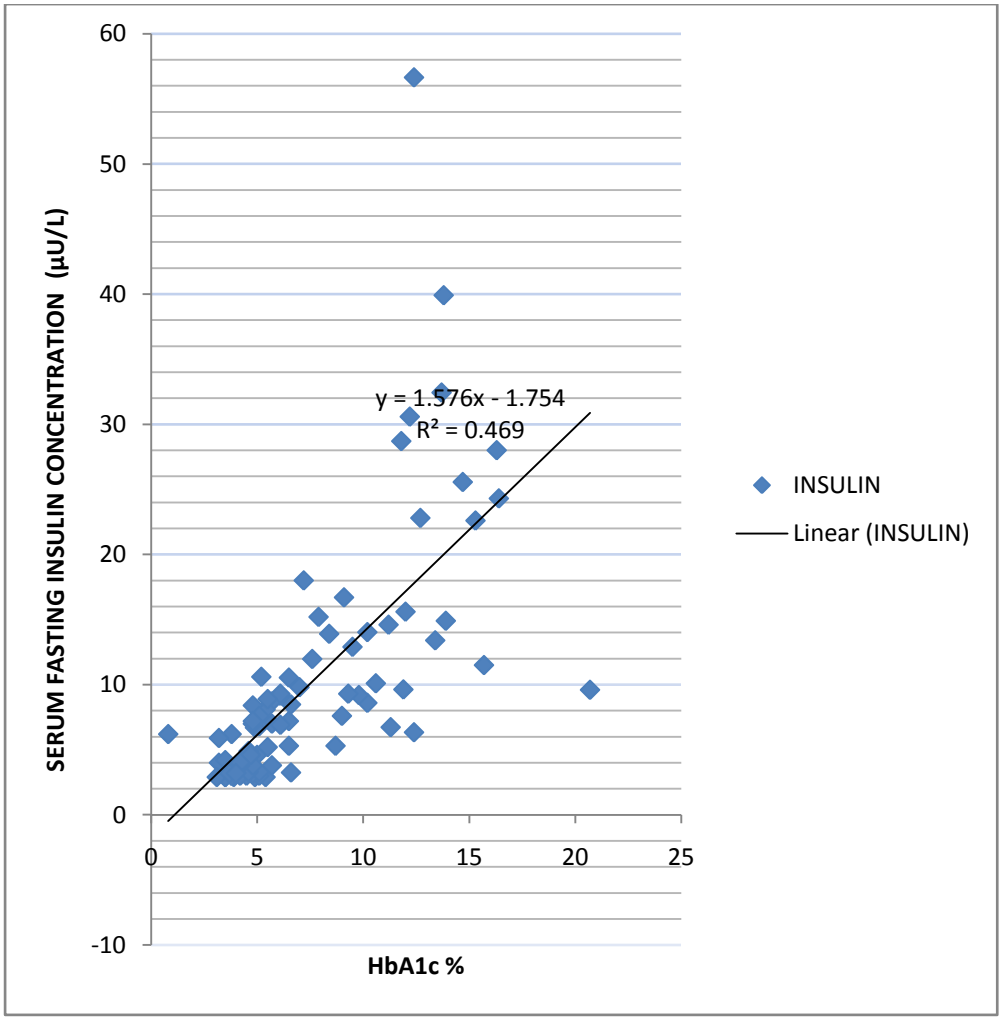


Figure 4.2: Relationship between fasting insulin and HbA1c levels in diabetic patients (r = +0.69, p = 0.0000)

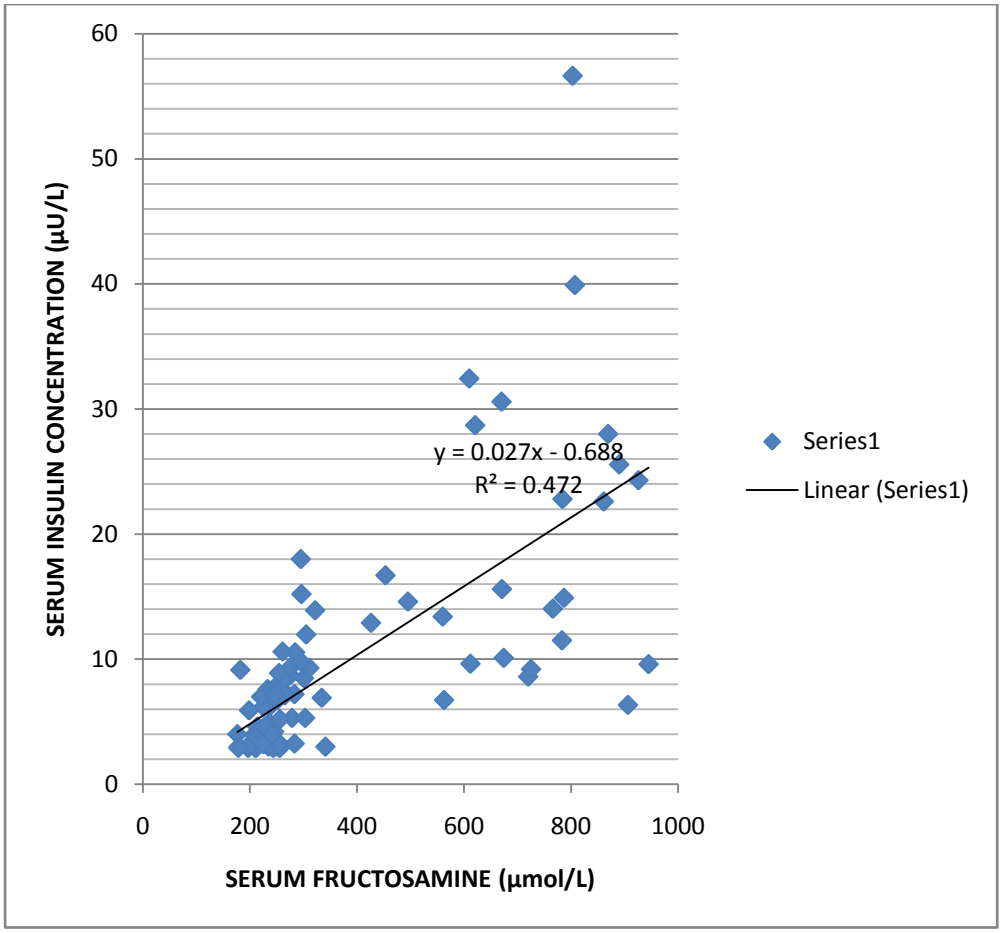


Figure 4.3: Relationship between fasting insulin and fructosamine levels in diabetic patients (r = + 0.69, p = 0.0001)

CHAPTER FIVE

5.1 DISCUSSION

This study showed the pattern of fasting serum insulin levels in diabetic patients and non diabetic healthy controls in Zaria and how it related with fasting blood glucose (FBG), glycated haemoglobin (HbA1c), fructosamine, body mass index (BMI) and blood pressure (BP).

The major findings of the present study were higher serum levels of insulin, fasting glucose, fructosamine and glycated haemoglobin in the diabetic patients than in non diabetic healthy controls. The serum insulin level and insulin resistance in diabetic patients increase significantly with increased body mass index which is not so in the controls. Serum insulin was significantly higher in diabetic patients with poor glycaemic control using any of the three markers of glycaemic control (FBG, fructosamine and HbA1c). There were linear relationships between insulin level and all the three markers of glycaemic control, that is fasting blood glucose, HbA1c or fructosamine levels in the diabetic patients.

The findings of higher insulin and body mass index (BMI) in diabetic patients than in controls in the current study suggest insulin resistance as the possible cause of diabetes mellitus in this environment. This is well supported by the finding of higher insulin resistance among diabetic patients than in controls. These findings agreed with those of others who reported that obesity causes insulin resistance, hyperinsulinaemia and diabetes mellitus (Williams *et al*, 1994) while this is contrary to the finding of Bakari*et al*, (2004) where the fasting insulin was significantly higher in the controls than in the diabetics. Other studies reported similar finding as Bakari*et al* (2004) that diabetes mellitus is associated with hypoinsulinaemia and pancreatic β – cell failure (Wicks *et al*, 1973; Omar *et al*, 1983). Hyperinsulinaemia in the present study may also be attributed to the effect of oral

antidiabeticdrugs prescribed to the patients whose main mechanism of action was to stimulate insulin synthesis and release (Frier*et al*, 1999; Inzucchi*et al*, 2012). High body mass index in diabetic patients could also be explained by the oral antidiabetic drugs administered by the patients with weight gain as part of their side effects especially sulphonylureas and thiazolidinediones (Frier*et al*, 1999; Inzucchi*et al*, 2012). Probably the pathophysiology of diabetes mellitus is changing in this environment from loss of beta cell function to insulin resistance. This could be explained by the higher BMI and higher insulin level in the diabetic patients than in the controls. This finding agrees with the explanation that the current rise in the prevalence of diabetes mellitus in United States was due to obesity, in fact it accounts for half of the increase in the prevalence in both men and women of the United States (Bonow et al, 2014). The present study observed the 2.5^{th} and 97.5^{th} percentiles of serum fasting insulin of 2.9 to 12.45 μIU/ml in healthy non diabetic control subjects. This is in contrast to the finding of fasting insulin level of 1.5 to 45 μIU/ml in healthy non diabetic controls by Bakari*et al* (2004). This pattern of change also suggests the rising insulin levels in diabetic patients probably due to increasing insulin resistance and not loss of beta cell function.

All the three markers of glycaemic control, that is, fasting blood glucose, HbA1c and fructosamine levels were significantly elevated in the diabetic patients than in the controls. This signifies poor glycaemic control among the diabetic patients. This may be due to lack of compliance to exercise, diet or drugs. This is consistent with the findings of Bakari*et al*, (2004) who reported in the same environment that diabetic patients had significantly elevated serum glucose than the controls.

There was no significant correlation between insulin, insulin resistance, FBG, HbA1c and fructosamine levels with BMI in the controls, while in the diabetic patients, insulin increased significantly with increase in BMI. Among diabetic patients, insulin resistance was

lowest among those with normal body mass index and was significantly higher in obese than in those with low and normal BMI. Rising obesity rates are the greatest contributor to the increasing prevalence of type 2 diabetes in the United States, accounting for all of the increase inwomen and about half of the increase in men (Bonow*et al*, 2014). Markers of glycaemic controls did not significantly relate with BMI in the diabetic patients. Bakari*et al* (2004) observed lack of correlation between insulin and BMI in the same environment. A positive correlation was observed between degree of obesity and insulin level in Pima Indians where prevalence of diabetes mellitus was up to 50 % but no correlation in Caucasians (Aronoff*et al*, 1977).

The insulin level in diabetic patients with good glycaemic control using fasting blood glucose of less than 7.0 mmol/L as the marker was significantly lower than in those with poor glycaemic control. Glycated haemoglobin of less than 7 % and fructosamineof less than 286 µmol/L were also used as markers of good glycaemic control and similar relationships were observed as with glucose. Bakari*et al* (2004) had made a similar observation when performing OGTT on diabetic patients and controls. He reported significant elevation in blood glucose levels with insulin.

There were linear and significant relationships between insulin and fasting blood glucose, insulin with HbA1c and insulin with fructosamine. Isah*et al* (1990) had related fasting blood glucose and fructosamine and found significant linear relationship in healthy Nigerian adults. Another observation by Frier*et al* (1999) demonstrated positive correlation between HbA1c and average fasting glucose. Although we cannot find a similar work between insulin levels and HbA1c, fructosamine and glucose, Isah*et al* (1990) and Frier*et al* (1999) demonstrated a possible linear relationship between the three markers of glycaemic control.

This study was able to relate body mass index and insulin level in diabetic patients and how the markers of glycaemic control relate with insulin in type 2 diabetic patients in Zaria. The possibility of obesity been a major contributor to rising incidence of diabetes mellitus rather than pancreatic β – cells failure as evidenced by hyperinsulinaemia in the current study should be a major concern to researchers, clinicians and policy makers to take a positive step towards that. Although, the sample size in the current study is low, it was able to provide some basic information about the pathophysiology of type 2 diabetes mellitus in Zaria and how insulin levels relate to the markers of glycaemic control in this group of patients. A larger study may be needed to determine the incidence/prevalence of diabetes mellitus, obesity and abnormal lipid profile and how they relate with insulin concentration and other markers of glycaemic control.

5.2 CONCLUSION

It can be concluded from the findings of the present study that:

1. Fasting serum glucose, insulin, HbA1c and fructosamine are significantly higher in diabetes than in controls.
2. As body mass index increases, the insulin and insulin resistance also increase in diabetic patients but not in controls, and this makes obesity and insulin resistance possible causes of diabetes mellitus in Zaria.
3. Fasting plasma glucose, HbA1c and fructosamine do no relate significantly with body mass index in both patients and controls.
4. Insulin level are lower in diabetic patients with good glycaemic control using any of the three markers that is FBG, HbA1c or fructosamine compared to diabetic patients with poor control.
5. There is linear relationship between fasting insulin level and the three markers of glycaemic control that is FBG, HbA1c and fructosamine.

5.3 RECOMMENDATIONS

Considering the findings of the present study, it can be recommended that:

1. Since serum fasting insulin level and insulin resistance correlate positively with the three markers of glycaemic control (serum fating glucose, HbA1c and fructosamine), they may be used as adjunct in the management of diabetes mellitus.
2. Clinicians should give emphasis on weight loss through regular exercise and diet while managing their diabetic patients since obesity is associated with increase in insulin resistance.
3. Insulin resistance, serum fasting insulin and glucose levels should be used to monitor response to therapy in diabetic patients.
4. Clinicians should also use HbA1c and fructosamine in the management of diabetic patients.

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APPENDIX I

THE RELATIONSHIP BETWEEN SERUM FASTING INSULIN CONCENTRATION AND MARKERS OF GLYCAEMIC CONTROL AMONG DIABETIC PATIENTS IN ABUTH, ZARIA.

CONSENT FORM

Serial No..... Hospital No…….. Date…….. Age .. Phone no:

This study is on 'Relationship between serum fasting insulin concentration and markers of glycaemic control among diabetic patients in ABUTH, Zaria'. It involves the collection of blood and urine samples from diabetic patients. The procedure involved will be fully explained to all participants. Each of the participants is free to participate or decline to participate without any consequence. The safety of all participants shall be given utmost priority. This study will hopefully determine the relationship between serum fasting insulin concentration and markers of glycaemic control among diabetic patients in ABUTH, Zaria. All participants will be given copies of their test results. The data from the study will be treated with strict confidentiality.

INFORMED WRITTEN CONSENT.

I……..of…….. (Address)

Have agreed to participate in this study. The full procedure before and after the test have been explained to me. I understand that a sample of my blood and urine will be collected. The results of these tests will be communicated to me to enable me take appropriate action with regards to my health.

I give this consent willfully without being subjected to any pressure.

Participants Name…… Signature……

Researcher's Name…… Signature……

Witness Name……. Signature……

APPENDIX II

HEALTH RESEARCH ETHICS COMMITTEE

AHMADU BELLO UNIVERSITY TEACHING HOSPITAL

SHIKA - ZARIA, NIGERIA.

E-mail: abuthshika@yahoo.com website: www.abuth.org

Chairman of Board: Chief. Shuaib Oyedokun Afolabi *Fnil*
Chief Medical Director: Prof. Lawal Khalid, *MBBS, FMCS, FWACS, FRCS(ED) mnl*
Chairman, Medical Advisory Committee: Prof. Abdullahi Mohammed, *MBBS, FWACP, FICS*
Director of Administration: Barr. Ishak Bello, *LL.B, BL., LL.M, PGDM, AHAN, FCAI*

Our Ref: ABUTH/HREC/TRG /36 Date: 19th Aug, 2015

Your Ref: _____

ABUTH HREC FULL ETHICAL CLEARANCE CERTIFICATE

Relationship between Serum Fasting Insulin Concentration and Markers of Glycaemic Control among Diabetic Patients in Ahmadu Bello University Teaching Hospital, Zaria.

ABUTH Ethics Committee assigned number:	-	ABUTHZ/HREC/N25/2015
Name of the principal Investigator:	-	Dr. Jibrin El-bashir,
Address of the Principal Investigator:	-	Dept. of Paediatrics, ABU Zaria,
Date of receipt of valid application:	-	14/4/ 2015
Date of meeting when final determination on ethical approval was made:	-	4th & 5th August, 2015

This is to inform you that the research described in the submitted protocol, the consent forms and other participant information materials have been reviewed and ***given full approval by the Health Research Ethics Committee.***

Please note: this approval dates from **20th August, 2015 -20th August, 2016**

No participant recruitment into this research may be conducted outside these dates.

All informed consent forms in this study must carry the ABUTH HREC number assigned to this research and the duration of ABUTH HREC approval of the study.

This HREC expects that you submit your application as well as an annual report for ethical clearance renewal 3 months prior to expiration of study dates. This is to enable you obtain renewal of your approval and avoid interruption of your research.

If there is delay in starting the research, please inform the ABUTH HREC so that starting dates can be adjusted accordingly.

No changes are permitted in the research without prior approval by ABUTH HREC, except in circumstances outlined in national code for Health Research Ethics: http://www.nhrec.net.

ABUTH HREC reserves the right to conduct compliance assessment visits to your research site without prior notification.

Prof. A. I. Mamman MBBS, FMCPath
Chairman, ABUTH HREC

pa

APPENDIX III
THE RELATIONSHIP BETWEEN SERUM FASTING INSULIN CONCENTRATION AND MARKERS OF GLYCAEMIC CONTROL AMONG DIABETIC PATIENTS IN ABUTH, ZARIA.
PROFORMA

SOCIO – DEMOGRAPHIC DATA

Serial number..... Hospital number…… Telephone number……..

Surname……. First name…… Middle name……..

Age…… Sex…… Highest educational level……..

Marital Status…….

MEDICAL HISTORY

Do you have the following?

a) Diabetes Mellitus Yes [ ] No [ ] if yes, for how long?.....years
b) Hypertension Yes [ ] No [ ] if yes, for how long?.....years
c) Stroke Yes [ ] No [ ] if yes, for how long?.....years
d) Low blood sugar requiring treatment. Yes [ ] No [ ] if yes, how many times……
e) Loss of consciousness due to high blood sugar. Yes [ ] No [ ] if yes, how many times…… When was the last episode?.....
f) Weight…….Kg Height……m BMI……..Kg/m^2
g) Abdominal circumference……..m Pelvic circumference…….m
   Abdomino – pelvic ratio……..
h) Drug/ Medication currently on:……
i) Fundoscopy findings……..

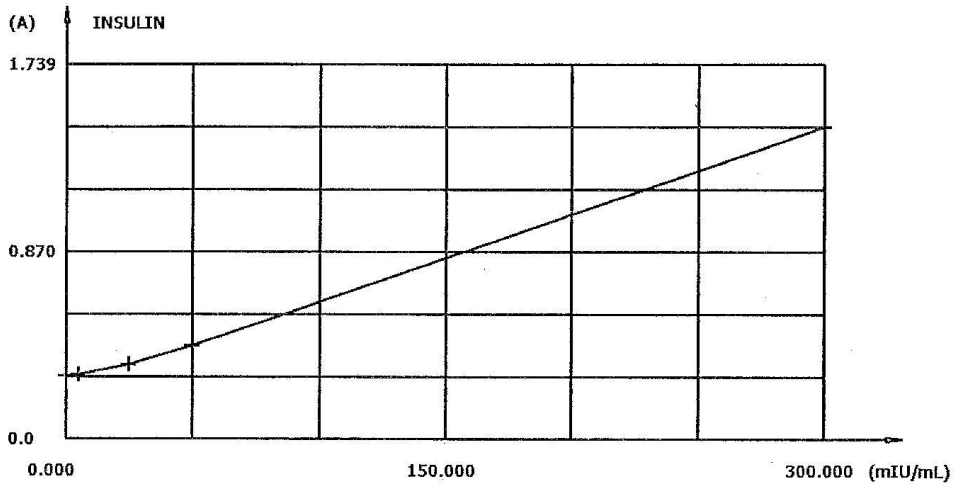
LABORATORY RESULT

1. Insulin
2. FBG
3. HbA1c
4. Fructosamine
5. hs CRP
6. Microalbuminuria
7. Total Cholesterol
8. HDL – Cholesterol
9. LDL – Cholesterol
10. Triglyceride

APPENDIX IV

Standard curve

RT-2100C



Program: INSULIN FASTING INSULICaculation: Curve Wavelength(nm): 450/630

Standard sample	#1	#2	#3	#4	#5
Con.(mIU/mL)	0.000	5.000	25.000	50.000	300.000
Abs(A)	0.291	0.299	0.343	0.433	1.449

Operator: Checker:
Print date: 11/25/2014