

**EVALUATION OF HBA1_c AS AN OBJECTIVE
MARKER FOR MONITORING BLOOD
GLUCOSE CONTROL FOR DIABETES
PATIENTS ON TREATMENT AT DORMAA
PRESBYTERIAN HOSPITAL**

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DECLARATION

The experimental work described in this thesis was carried out at the laboratory Department of Dormaa Presbyterian Hospital, Dormaa Ahenkro. This work has not been submitted for any other degree.

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ABSTRACT

The unavailability or the expensiveness of new technologies such as glycosylated haemoglobin test to monitor long term glycaemic control is a problem in the Dormaa municipality. This study was aimed at evaluating the relationship between glycosylated haemoglobin (HbA1c) concentration and levels of instant fasting plasma glucose (FPG) concentration and mean fasting plasma glucose (FPG) concentration over a three-month period of diabetes patients on treatment. Hundred and fifty whole and plasma samples were collected from systematic grouping of diabetics on treatment (100) attending diabetes clinic at Dormaa Presbyterian hospital as consistently hypoglycaemic $<3.5\text{mmol/l}$ (20), consistently normal $3.5\text{--}5.9\text{ mmol/l}$ (20),consistently above normal $6\text{--}6.9\text{mmol/l}$ (30) and hyperglycaemic $\geq 7.0\text{ mmol/l}$ (30) and 50 non diabetics healthy people in Dormaa Ahenkro, in Ghana. All laboratory assays were performed according to reagent and equipment manufacturers' standards. HbA1c assays were measured by DCA 2000 immunossay method, FPG were performed using glucose hexokinase method and haemoglobin levels (Hb) were assayed using Cyanmethaemoglobin assay. Socio-demographic data of all eligible participants was captured using a structured questionnaire. On the average, the diabetics ($49.28\pm1.42\text{ yrs}$) and the non-diabetics ($48.98\pm2.92\text{ yrs}$) were age matched. The mean systolic blood pressure of the diabetes group and its systematic groupings were significantly higher ($p < 0.001$) as compared to the control group. However there were no significant differences between diastolic pressures for both diabetics and non-diabetics. Even though the mean fasting plasma glucose of the diabetic group (5.17 ± 0.19) was comparable to that of the control group (5.17 ± 0.079) $p>0.05$, the levels of HbA1c among the diabetics were significantly higher as compared to the control group. There was significant positive correlation between HbA1c and the various monthly instant fasting plasma parameters as well as the mean fasting plasma glucose. It was observed that the strongest positive linear

correlation existed between the HbA1c concentration and the average FPG concentration for the three-month period. Mean glycaemia strongly correlates with HbA1c be reliably depended on as an index of prognosis of diabetes mellitus. During treatment, for good prognosis the goal must be to keep HbA1c less than 7%

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TABLE OF CONTENTS

DECLARATION	I
ABSTRACT.....	II
ACKNOWLEDGEMENT.....	IV
TABLE OF CONTENTS.....	V
LIST OF TABLES.....	VIII
LIST OF FIGURES.....	IX
CHAPTER 1 INTRODUCTION	1
1.1 GENERAL INTRODUCTION	1
1.2 JUSTIFICATION.....	3
1.3 AIMS AND OBJECTIVES	4
CHAPTER 2 LITERATURE REVIEW.....	1
2.1 DIABETES MELLITUS	1
2.2 METABOLIC DISTURBANCES IN DIABETES.....	4
2.3 LONG-TERM COMPLICATIONS OF DIABETES	5
2.3.1 <i>Metabolic control and development of long term complications</i>	6
2.3.2 <i>Protein Glycation</i>	7
2.4 DIAGNOSIS OF DIABETES MELLITUS.....	8
2.4.1 <i>Blood Glucose</i>	8
2.5 LABORATORY INTERVENTIONS IN BLOOD GLUCOSE CONTROL.....	9
2.5.1 <i>Glycosylated Haemoglobin</i>	9
2.5.1.1 Nonenzymatic glycation, structural insights and chemical studies..	12
2.5.1.2 Methodologies to estimate HBA1c	15
2.5.1.3 Methods based on differences in ionic charge	16
2.5.1.4 Methods based on structural characteristics of HBA1c	16
2.5.1.5 Methods based on chemical reactivity	17
2.5.1.6 Targets for patients with diabetes.....	18
2.5.1.7 HBA1c as a screening test for diabetes	19
2.5.1.8 Frequency of HBA1c testing	21
2.5.1.9 Importance of HBA1c.....	22
2.5.1.10 Factors affecting HbA1c	23
2.5.1.11 Blood Sugar.....	23

2.5.1.12	Red cell survival.....	24
2.5.1.13	Iron deficiency anaemia	24
2.5.1.14	Blood transfusion.....	24
2.5.1.15	Haemoglobin variants	24
2.5.1.16	Uraemia	25
2.5.1.17	Vitamins C and E	26
2.5.1.18	Hypertriglyceridaemia	26
2.5.1.19	Aspirin	27
2.5.1.20	Race	27
2.5.1.21	Relaxation.....	27
2.5.1.22	Glycated serum proteins	27
2.5.1.23	Self monitoring of blood glucose (SMBG).....	28
2.5.1.24	Urine testing	30
CHAPTER 3	MATERIALS AND METHODS	31
3.1	AREA OF STUDY STATISTICS	31
3.2	STUDY DESIGN	32
3.3	STATISTICAL METHODS	32
3.4	SUBJECT SELECTION	33
3.4.1	<i>Inclusion Criteria.....</i>	33
3.4.2	<i>Exclusion criteria.....</i>	35
3.5	SAMPLE COLLECTION.....	35
3.5.1	<i>Materials for fasting plasma glucose estimation</i>	36
3.5.1.1	Methodology: Blood Glucose Estimation.....	37
3.5.1.2	Precautions In Glucose Assay	38
3.5.1.3	Reagent preparation.....	38
3.5.1.4	Reagent storage	38
3.5.1.5	Storage and stability	38
3.5.2	<i>Methodology For Glycated Haemoglobin (HbA1c) Estimation.....</i>	39
3.5.2.1	Reagents.....	39
3.5.2.2	Principle Of The Measurement Of HbA1c.....	40
3.5.2.3	Procedure For Glycated Haemoglobin Measurement	41
3.5.3	<i>Methodology for haemoglobin estimation</i>	43
3.5.3.1	Materials	43
3.5.3.2	Reagents :.....	43

3.5.3.3 Procedure For Haemoglobin Concentration Measurement	44
CHAPTER 4 RESULTS	46
4.1 Anthropometric, clinical and laboratory characteristics of the entire study group (diabetics and non diabetics (controls) as stratified by fasting plasma glucose.....	46
4.2 Pearson product correlation between anthropometric, clinical and laboratory indices of female and male diabetic subjects.....	49
4.3 Correlation between HbA1c and various monthly fasting plasma glucose as well as that between mean fasting plasma glucose of the diabetic group	
4.4 Correlation analysis between average glycosylated haemoglobin and anthropometric and clinical variables for diabetic group.....	53
4.5 Correlation coefficient with HbA1c	53
4.6 Correlations between HbA1c and anthropometric and clinical variables of non diabetics	54
4.7 for non diabetics (controls)	54
4.8 Correlation coefficient between mean fasting plasma glucose and anthropometric and clinical variables of diabetics.....	54
4.9 Correlation coefficient with FPG	55
4.10 Blant–altman comparison of HbA1c among glycaemic levels in diabetics and non diabetics.....	55
4.11 Relative sensitivity and specificity of HbA1c among diabetics and non diabetic subjects	56
4.12 Linear regression between independent variable blood glucose and dependent variable glycated haemoglobin	59
CHAPTER 5 DISCUSSION, CONCLUSION & RECOMMENDATION.....	61
5.1 DISCUSSION	61
5.2 CONCLUSION	73
5.3 RECOMMENDATION.....	74
REFERENCES	75
APPENDIX.....	ERROR! BOOKMARK NOT DEFINED.

LIST OF TABLES

Table 4-1: Anthropometric, Clinical And Laboratory Characteristics Of Diabetic Subjects and Non Diabetic Controls Stratified By Fasting Plasma Glucose Levels.....	48
Table 4-2: Pearson Product Correlation Coefficient between Anthropometric Indices, Clinical Variables and Laboratory Measurements: Female Diabetics (Upper Right- Hand Side) and Male Diabetics (Lower Left-Hand Side).....	51
Table 4-3: Pearson Product Correlation Coefficient between monthly fasting plasma glucose and glycated haemoglobin (HbA1c), as well as that between mean fasting plasma glucose.	52
Table 4-4: Correlation of HbA1c versus anthropometric and clinical variables of diabetics.....	53
Table 4-5: Correlation of HbA1c versus anthropometric and clinical variables of controls	54
Table 4-6: Correlation of Mean FPG against anthropometric and clinical variables of diabetics	55
Table 4-7: Bland Altman comparison of HbA1c among different glycaemic levels	56

LIST OF FIGURES

Figure 2-1: Source : Diabetes Management by Aventis, 2006 showing the formation of Glycosylated haemoglobin molecule	11
Figure 2-2: A Chart showing HbA1c and Mean Blood Glucose Comparison (Source: Embee Diagnostics, 1996)	19
Figure 3-1: Bayer DCA 2000 analyzer	42
Figure 4-1: Relative Sensitivity And Specificity of HbA1c Among Diabetics And Non Diabetic Subjects.....	58
Figure 4-3: Linear Regression Graphs of HbA1c versus Monthly Fasting Plasma Glucose.....	60

Chapter 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

Diabetes mellitus is a chronic metabolic disease characterized by dysmetabolism including hyperglycaemia resulting from defects in insulin secretion, insulin action, or both (**Reaven, 1988**). Uncontrolled chronic hyperglycaemia results in long-term damage, particular dysfunction, and involvement of the eyes, heart, blood vessels, nerves, and kidneys. Diabetes is a serious metabolic disease that results in the body not properly processing glucose in the blood (**WHO, 1998**). The global prevalence of diabetes mellitus among adults was estimated to about 150 million in 1995, and this was projected to increase by 2025 to 300 million people (**Aubert *et al*, 1998**). Estimates from the World Health Organization (WHO) are similar, projecting an increase from 171 million in 2000 to 366 million in 2030 (Green *et al*, 2004). Diabetes is becoming a major health problem in Ghana and over 90% of these are non-insulin dependent Diabetes Mellitus (NIDDM Type 2). The prevalence of this condition was reported to be 0.2-0.4% in 1976 (**Owusu, 1976**). This figure is thought to have risen dramatically over the years. Prevalence estimate of Diabetes mellitus in Ghana was 6.3% in 2003 (**Amoah *et al*, 2002**).

The goal of diabetes management is the prevention of acute and chronic complications of diabetes mellitus. Traditional complications of diabetes are viewed as the micro vascular complications including retinopathy, neuropathy and nephropathy. However, the macro vascular complications of diabetes are more

prevalent and are the major cause of diabetes disability and death in diabetic population (**Gill, 1997**). The core of the issue is glycaemic control. It has long been suspected that high blood glucose is harmful in a variety of ways and that all the complications whether microvascular or macrovascular, were to a larger or lesser extent linked with it. In recent times this has been well established. Amongst the various markers of glycaemic control, glycosylated haemoglobin (GHb) has now been established as the most reliable, though many other proteins are also glycated in the diabetic and non-diabetic states (**WHO, 2003**). Glycosylated haemoglobin (HbA1c) is a reliable index of glycaemic control in permitting appropriate changes in treatment and identifying inconsistency with the patient's record of instant blood glucose monitoring. HbA1c is also an index to assess the effectiveness of therapy by monitoring long-term serum glucose regulation (**Cahill *et al*, 1976**).

In this study the focus is on the use of glycated haemoglobin measurement for monitoring diabetes in the long term as mean glycaemic control instead of using only instantaneous fasting plasma glucose. Measurements of glycosylated haemoglobin and fasting plasma glucose in blood play a key role in the investigation and treatment of people with diabetes (**Alexandria, 1996**). Appropriate testing varies widely according to the clinical presentation, physical findings, and results of other diagnostic procedures. In general; the most important glycaemic parameter measured is glycated haemoglobin (HbA1c) from all participants. The anthropometric, clinical and laboratory parameters measured

were done stratified by glucose levels as hypoglycaemia, low normal, high normal and hyperglycaemia.

1.2 JUSTIFICATION

The aim of treatment is to achieve as near normal metabolism as is practicable. Poor glycaemic control refers to persistently elevated blood glucose and glycosylated haemoglobin levels (**Alexandria, 1996**). The nearer the body weight approaches the ideal level and the closer the blood glucose concentration is kept to normal, the more the total metabolic profile is improved and the lower the incidence of vascular disease and specific diabetic complications(**Alexandria, 1996**). Currently laboratory tests used to monitor diabetes mellitus include fasting plasma glucose, urine glucose, urine ketones tests, serum electrolytes, urea and creatinine. While instant fasting plasma glucose provides useful information for day-to-day management of diabetes, an objective measure of glycaemia over an extended period of time is also required. This is achieved by the measurement of glycated proteins which can estimate average glycaemia over months (**Sacks *et al*, 2002**). Although studies have shown a close relationship between the concentrations of HbA1c and mean glycaemia, routine determinations of blood glucose by patients or by their healthcare providers are not considered as reliable as HbA1c to quantify mean glycaemia (**British Medical Journal, 1980**). Health workers hardly request glycosylated haemoglobin test and diabetics have not been educated on the need to monitor long term control of diabetes. There is paucity of

information on the correlation between HbA1c and instant fasting glucose as well as mean fasting glucose, demographic, anthropometric and clinical variables in the Ghanaian populace.

1.3 AIMS AND OBJECTIVES

- 1) To determine if there are any significant difference in the demographical, anthropometric, clinical and laboratory profiles of diabetics and control group as stratified by blood glucose.
- 2) To investigate how well glycosylated haemoglobin (HbA1c) test correlates with instant fasting plasma glucose (FPG) concentration and mean FPG concentration over a three-month period.
- 3) To evaluate how well glycosylated haemoglobin (HbA1c) test correlates with anthropometric and clinical indices
- 4) To determine the relationship between mean fasting plasma glucose test, clinical and anthropometric indices.

Chapter 2

LITERATURE REVIEW

2.1 DIABETES MELLITUS

Diabetes mellitus is a clinical syndrome characterised by dysmetabolism including hyperglycaemia due to absolute or relative deficiency of insulin. This can arise in many different ways (**Stryer, 1995**). Lack of insulin affects the metabolism of carbohydrate, protein and fat, and causes a significant disturbance of water and electrolyte homeostasis (**Henry *et al*, 1974**). Death may result from acute metabolic decompensation, while long-standing metabolic derangement is frequently associated with permanent and irreversible functional and structural changes in the cells of the body, with those of the vascular system being particularly susceptible (**Baynes *et al*, 1984**). These changes lead to the development of well-defined clinical entities, the so-called 'complications of diabetes' which characteristically affect the eye, the kidney and the nervous system (**King and Rewers, 1993**). Epidemiological studies of whole populations have shown that the distribution of blood glucose concentration is unimodal, with no clear division between normal and abnormal values. However, hyperglycaemia represents an independent risk factor for the development of disease of both small and large blood vessels (**Chatured *et al*, 1996**). Diagnostic criteria for diabetes have therefore been selected on the basis of identifying those who have a degree of

hyperglycaemia which, if untreated, is associated with a significantly increased risk of developing vascular disease. The implication of these criteria is that there is no such thing as 'mild' diabetes not requiring effective treatment. The global burden of disease study of the World Health Organization (WHO) estimated that about 177 million people in the world had diabetes in the year 2000 **(WHO, 2003)**.

In the second edition of the International Diabetes Federation's Diabetes Atlas it is estimated that 194 million people had diabetes in the year 2003, and about two-thirds of these people lived in developing countries **(IDF 2003)**. Communicable diseases still make up the greatest disease burden, but by 2020, noncommunicable diseases, including hypertension and diabetes, will outstrip communicable diseases as a cause of death **(Murray and Lopez 1997)**. This situation is a result of demographic change (populations with older age structures), increasing urbanization **(WHO ,1998)** and associated changes in risk-factor levels, such as tobacco smoking, obesity, and physical inactivity **(Hunter *et al*, 2000)**. Countries of sub-Saharan Africa are in various stages of the epidemiological transition with a multiple burden of diseases. The available evidence suggests that noncommunicable diseases currently contribute substantially to the burden of mortality and morbidity in adults. Age-specific levels of diabetes and hypertension in many urban areas of sub-Saharan Africa are as high as, or higher than, those in most Western European countries **(Hunter *et al*, 2000)**. Hypertension is present in 50% of patients with type 2 diabetes.

Diabetes mellitus can be classified into four principal types (**WHO, 1998**). This includes type 1 diabetes, type 2 diabetes, other specific types of diabetes, and gestational diabetes mellitus. The most common types of diabetes seen in sub-Saharan Africa are type 1 and type 2 diabetes mellitus.

Type 1 diabetes results from autoimmune destruction of the pancreatic beta cells, causing the loss of insulin production. Children are usually affected by this type of diabetes, although it occurs at all ages and the clinical presentation can vary with age. Patients with this type of diabetes require insulin for survival (**WHO, 1998**).

Type 2 diabetes is characterized by insulin resistance and abnormal insulin secretion, either of which may predominate but both of which are usually present. The specific reasons for the development of these abnormalities are largely unknown. Type 2 is the most common type of diabetes. Type 2 diabetes can remain asymptomatic for many years, and the diagnosis is often made from associated complications or incidentally through an abnormal blood or urine glucose tests (**World Health Organization, 2010**). Other specific types of diabetes include those due to genetic disorders, infections, endocrinopathies, and drugs. This last type of diabetes is relatively uncommon.

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. The definition applies whether insulin or only diet modification is used for treatment and whether the condition persists after pregnancy. It does not exclude the possibility that

unrecognized glucose intolerance may have antedated or begun concomitantly with the pregnancy. Approximately 7 percent of all pregnancies are complicated by GDM. The prevalence may range from 1 to 14 percent of all pregnancies, depending on the population studied and the diagnostic tests employed **(Alexandria, 1996)**.

Impaired glucose tolerance (IGT) is asymptomatic, and its diagnosis is confirmed by an elevated nondiabetic level of blood glucose two hours after a 75 gram oral glucose tolerance test. Impaired fasting glycaemia (IFG) is an elevated nondiabetic fasting blood glucose level. Both IGT and IFG are transitional stages in the development of type 2 diabetes **(Alexandria, 1996)**.

2.2 METABOLIC DISTURBANCES IN DIABETES

The hyperglycaemia of diabetes develops because of an absolute (type 1 diabetes) or a relative (type 2 diabetes) deficiency of insulin, resulting in decreased anabolic and increased catabolic effects **(Stanley *et al*, 1982)**. In both type 1 and type 2 diabetes, the actions of insulin are also impaired by insensitivity of target tissues. While this is a fundamental defect in type 2 diabetes, hyperglycaemia can also reduce insulin secretion by the effect of glucose toxicity on beta cell function. Glycosuria occurs when the plasma glucose concentration exceeds the renal threshold (the capacity of renal tubules to reabsorb glucose from the glomerular filtrate) at approximately 10 mmol/l **(Ravel, 1984)**. The severity of the classical osmotic symptoms of polyuria and polydipsia is related to the degree of glycosuria. If hyperglycaemia develops slowly over months or years, as in type 2

diabetes, the renal threshold for glucose rises, and the symptoms of diabetes are mild. This is one reason for the large number of undetected cases of type 2 diabetes, many of which are discovered coincidentally (**American Diabetes Association, 2003**).

2.3 LONG-TERM COMPLICATIONS OF DIABETES

The long-term results of treatment of diabetes are disappointing in many patients. The excess mortality incurred by diabetic patients is mainly caused by large blood vessel disease, which accounts for about 70% of all deaths, mostly from myocardial infarction and stroke (**Boule *et al*, 2003**). The pathological changes associated with atherosclerosis in diabetic patients are similar to those seen in the non-diabetic population but they occur earlier in life and are more extensive and severe. Diabetes enhances the effects of the other major cardiovascular risk factors: smoking, hypertension and hyperlipidaemia. Hyperinsulinaemia may promote atherogenic changes in blood lipids and blood coagulability and raise arterial blood pressure. A metabolic (insulin resistance) syndrome has been described in which the co-segregation of various conditions is associated with premature and severe macrovascular disease. However, randomized controlled trials have shown that aggressive management of diabetic patients with cardiovascular disease can improve outcome. Disease of small blood vessels is a specific complication of diabetes and is termed diabetic microangiopathy. It contributes to mortality by causing renal failure due to diabetic nephropathy (**Tuomilehto *et al*, 2001**).

Both types of vascular disease also cause substantial morbidity and disability: for example, blindness due to diabetic retinopathy; difficulty in walking, chronic ulceration of the feet, bowel and bladder dysfunction due to autonomic neuropathy; and angina, cardiac failure, intermittent claudication and gangrene due to atherosclerosis (Tuomilehto *et al*, 2001).

2.3.1 Metabolic control and development of long term complications

A granted relationship has been demonstrated between the duration and degree of sustained hyperglycaemia, however caused and at whatever age it develops, and the risk of vascular disease. The possibility of reversing early vascular disease by improving metabolic control has been examined in several prospective randomized controlled clinical trials involving patients with early background retinopathy and minimal proteinuria. None of these studies produced any evidence of reversal of either retinopathy or nephropathy, and in some cases retinopathy worsened abruptly soon after control was improved. Despite this, the long term rate of progression of both retinopathy and nephropathy was reduced by continuing better control. These studies stimulated a search for markers of early reversible retinal, renal and neural dysfunction, and shifted the emphasis in the management of diabetes to primary prevention of complications (**Diabetes Control and Complications Trial, DCCT, 1993**).

The Diabetes Control and Complications Trial (**DCCT, 1993**) was a large study that lasted 9 years in type 1 diabetic patients to answer the question: are diabetic complications preventable? The trial demonstrated a 60% overall reduction in the

risk of developing diabetic complications in those on intensive therapy with strict glycaemic control (mean HbA_{1c} around 7%), compared with those on conventional therapy (mean HbA_{1c} around 9%). No single factor other than glycaemic control had a significant effect on outcome DCCT Research Group.

A large study of patients with type 2 diabetes, U.K. Prospective Diabetes Study (UKPDS, 1998), has shown that the frequency of diabetic complications is lower and progression slower with good glycaemic control and effective treatment of hypertension, irrespective of the type of therapy used. This study has indicated that the target, HbA_{1c} should be 7% or less and blood pressure less than 140/80. This often requires the use of multiple medications, with the potential problem of patient adherence to therapy.

2.3.2 Protein Glycation

The entry of glucose into the brain, peripheral tissues, kidney, intestines, lens and red blood cells does not depend on insulin action (Brown, 1990). Consequently; during hyperglycaemia the intracellular level of glucose in these cells is high. This promotes the nonenzymatic attachment of glucose to protein molecules (protein glycation) (Koenig, 1976). During the 1970s and 1980s, it was realized that this process, called the Maillard reaction or advanced glycation, also occurs slowly *in vivo*. Advanced glycation endproducts (AGEs) that form are implicated, causing the complications of diabetes and aging, primarily via adventitious and crosslinking of proteins. Long-lived proteins such as structural collagen and lens crystallins particularly are implicated as pathogenictargets of AGE processes

(Diabetes Control and Complications Trial Research Group 1993). AGE formation in vascular wall collagen appears to be an especially deleterious event, causing crosslinking of collagen molecules to each other and to circulating proteins. This leads to plaque formation, basement membrane thickening, and loss of vascular elasticity. The chemistry of these later-stage, glycation-derived crosslinks is still incompletely understood but, based on the hypothesis that AGE formation involves reactive carbonyl groups, the authors introduced the carbonyl reagent aminoguanidine hydrochloride as an inhibitor of AGE formation *in vivo* in the mid 1980s. Subsequent studies by many researchers have shown the effectiveness of aminoguanidine in slowing or preventing a wide range of complications of diabetes (Goldstein *et al*, 2004).

2.4 DIAGNOSIS OF DIABETES MELLITUS

2.4.1 Blood Glucose

This can be determined in blood samples collected into heparinised tubes containing sodium fluoride as an inhibitor of glycolysis. Modern laboratory tests employ enzymatic reactions that provide measurements over a broad range of glucose concentration by colorimetry. The (WHO, 1997) has published guidelines to the diagnosis of diabetes mellitus on the basis of blood glucose results and response to an oral glucose load. The values are based on the threshold for risk of developing vascular disease. Diabetes is defined by fasting plasma glucose of 7.0 mmol/l or above, or random plasma glucose of 11.1 mmol/l or above, or an

abnormal oral glucose tolerance test. Intermediate readings on the glucose tolerance test are classified as 'impaired glucose tolerance' (IGT) and indicate the need for further evaluation. Another abnormal finding is 'fasting hyperglycaemia' or 'impaired fasting glucose', when the fasting plasma glucose is between 6.1 and 6.9 mmol/l. Patients with impaired fasting glucose have an increased risk of developing vascular disease. Reliance on fasting blood glucose values alone, as advocated by the American Diabetes Association, will miss some cases of type 2 diabetes which are revealed by the oral glucose tolerance test (**Alexandria, 1996**).

2.5 LABORATORY INTERVENTIONS IN BLOOD GLUCOSE CONTROL

2.5.1 Glycosylated Haemoglobin

Haemoglobin A1c was first separated from other forms of hemoglobin by Huisman and Meyering in 1958 using a chromatographic column (**Huisman *et al*, 1958**). It was first characterized as a glycoprotein by Bookchin and Gallop in 1968 (**Bookchin and Gallop, 1968**). Its increase in diabetes was first described in 1969 by Samuel Rahbar and coworkers (**Rahbar, 1969**). The reactions leading to its formation were characterized by Bunn and his co-workers in 1975 (**Bunn *et al*, 1975**). The use of haemoglobin A1c for monitoring the degree of control of glucose metabolism in diabetic patients was proposed in 1976 by Anthony Cerami, Ronald Koenig and coworkers (**Koenig *et al*, 1976**). Human haemoglobin, in its structural and functional features, is the most extensively studied protein. Glycosylated haemoglobin (HbA1c) refers to substances which are formed by any carbohydrate

binding to haemoglobin in the red blood cell. Glycohaemoglobin is also used as an alternative term to glycosylated haemoglobin.

The major forms of adult haemoglobin (Hb) are:

- HbA (which consists of 2α and 2β chains) and makes up about 90% of adult Hb and includes:
 - HbA0 – is the non-glycated fraction of HbA and
 - HbA1 – is the glycated fraction of HbA which has the following subfractions:
 - HbA1a1 – in which fructose-1, 6-biphosphate is bound to the β chain
 - HbA1a2 – in which glucose-6-phosphate is bound to the β chain
 - HbA1b – in which unknown carbohydrate is bound to the β chain
 - HbA1c – in which glucose is bound to the β chain
 - HbA2 (which consists of 2α and 2δ chains) and makes up about 2.5% of adult Hb
 - HbF (foetal haemoglobin) consists of 2α and 2γ chains and is the major form of foetal haemoglobin and almost completely disappears within 6 months of birth and makes up < 1% of adult Hb.

The HbA1c fraction is the major part of glycated haemoglobin and is formed by the binding of glucose to the N valine terminal of the β chain of Hb. This occurs in a two-step process. The initial and rapid process takes minutes to hours to form an

aldimine complex (Schiffbase), a reaction which is reversible. Over subsequent days to weeks this unstable aldimine complex undergoes an Amadori rearrangement to form the stable ketoamine HbA1c. Glucose binding (glycation) occurs slowly and continuously over the life span of a red blood cell (120 days) (**Bunn *et al*, 1978**). Because erythrocytes are freely permeable to glucose, the level of HbA1c provides a glycaemic history of the previous 120 days, the average erythrocyte lifespan. HbA1c reflects the time averaged blood glucose over the preceding 1-3 months (**Bunn *et al*, 1978**) and is highly correlated to long-term complications of diabetes (retinopathy, nephropathy and neuropathy). Glycosylated haemoglobins, known to have an increased affinity for oxygen when purified and in diluted solutions, do not play a significant role in the oxygen affinity pattern of diabetics at the concentrations normally found in vivo (**Monnier *et al*, 2003**).

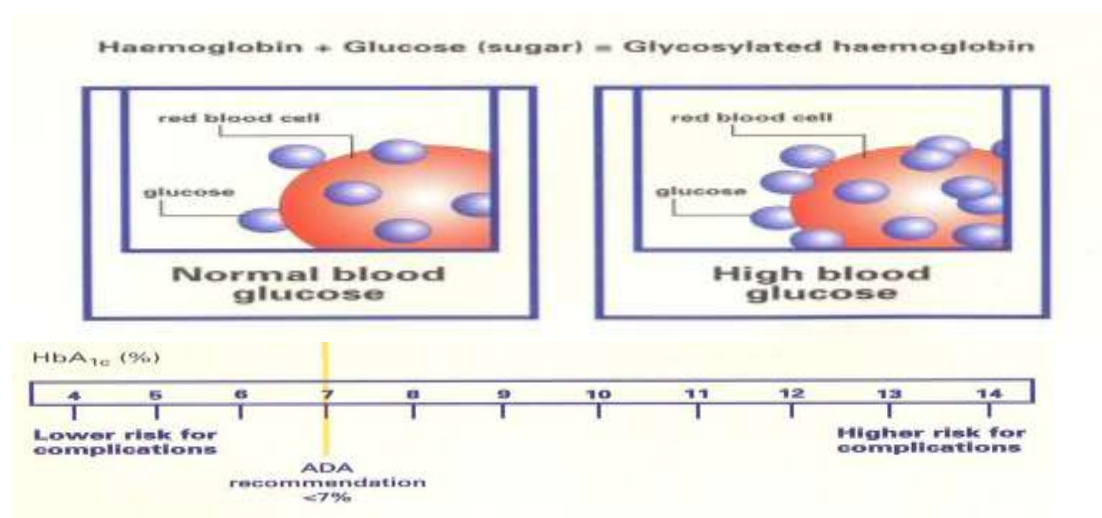


Figure 2-1: Source: Diabetes Management by Aventis, 2006 showing the formation of Glycosylated haemoglobin molecule

2.5.1.1 Nonenzymatic glycation, structural insights and chemical studies.

Of the several pathogenic mechanisms by which hyperglycemia may lead to altered tissue structure and function, non-enzymatic glycation (encompassing the attachment of the free aldehyde groups of glucose or other sugars to the non-protonated free amino groups of proteins) changes the structure and function of several soluble and insoluble proteins *in vivo* and *in vitro*) (**Lyons et al, 1991**). Because cells and their extracellular matrix share a dynamic and reciprocal relationship, modulations of matrix components by glycation leads to altered cell behaviour, including changes in cell spreading, phosphorylation of key intracellular signaling molecules, and expression of extracellular matrix proteins and their modulators (**Striker et al, 1993**). Extracellular matrix from diabetic patients is more extensively glycated than extracellular matrix from nondiabetic people. In addition, the accumulation of glycation products and the accompanying structural extracellular matrix modifications correlate with the development of functional complications of diabetes (**Monnier et al, 1986**). These changes in tissue structure and function are slow and cumulative, producing a long time lag between the start of diabetes and the onset and progression of the complications. Earlier chromatographic studies indicated that HbA1c was identical to HbA, with an identified group linked to the NH₂-terminal valine of the *b*-chain; that such modification was possible through a Schiff's base was indicated by its reduction by borohydride (**Holmquist and Schroeder, 1966**). Analysis by mass spectrometry of the blocking group by the same authors pointed to a hexose linked to NH₂-terminal valine, with no clue then to its structure or nature of linkage. Later, mild

acid hydrolysis of HbA1c yielded glucose and mannose in a 3 : 1 ratio, and a total yield of 20–30%. Elegant experimental evidence proved that in the red cell, glucose reacts first with the NH₂-terminal of *b*-chain to form an aldimine linkage, next undergoing an Amadori rearrangement to form the more stable ketoamine linkage, came by using tritiated borohydride reduction and subsequent oxidation with periodate of the isolated *b*-chain, containing 95% of the radioactivity (**Bunn *et al*, 1975**). Nearly all the radioactivity was recovered as 3H formic acid, rather than formaldehyde, strongly suggestive that the second carbon atom of glucose was tritiated rather than the first. Mechanistically therefore, in the red cell, glucose forms an aldimine linkage with NH₂- of valine of the *b*-chain, undergoing an Amadori rearrangement to form the more stable ketoamine linkage as shown in Figure 2.

One can thus explain on the basis of racemization at the second carbon atom during acid hydrolysis, the C-2 epimer of glucose, viz. mannose being formed. Treatment of HbA1c with mild acid results (by promoting the dehydration of the sugar group) in the formation of 5-hydroxymethylfurfural (5-HMF). These mechanisms were promptly confirmed and conditions determined for the establishment of a colorimetric method of utility for the determination of HbA1c.





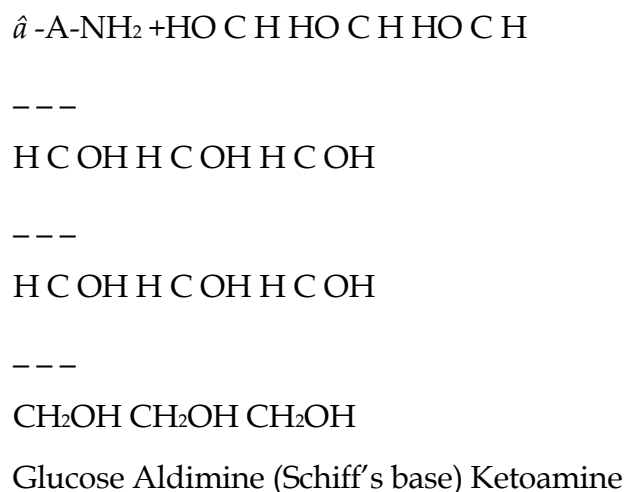


Figure 2. Amadori rearrangement of glucose molecule (**Chandalia, *et al*, 1998**).

Biosynthesis of glycosylated haemoglobins (HbA1a, HbA1b, and HbA1c) occurs slowly, continuously and almost irreversibly throughout the four month life span of erythrocytes and the process is wholly non-enzymatic, as demonstrated by elegant human studies using **Fe**-bound transferrin and measurement of specific radioactivity of the major and minor haemoglobin components during the entire life span of erythrocytes. Sugar phosphates, such as glucose-6-phosphate (G-6-P) present in red cells, can react with haemoglobin 20 times faster than glucose, in fact, with greater specificity than glucose. Fructose-6-phosphate, fructose-1, 6-diphosphate, ribose-5-phosphate, ribulose 5-phosphate, and glucuronic acid but not glucose-1-phosphate or glucose 1,6-diphosphate react with haemoglobin with the rapid formation of the adduct, thus requiring an aldehyde or ketone group separated from a negatively charged COO^- or PO_4^{3-} group. It is very unlikely that G-6-P haemoglobin is a precursor of HbA1c. Concentration of G-6-P in red cells is 1/200th that of glucose, but G-6-P reacts with haemoglobin ten times more rapidly

than glucose. Structure–function relationship can be studied with considerable significance on both natural and synthetic derivatives, on account of the specificities in relation to sites of glycation. Studies on the role of potentially catalytic residues on the polypeptide (protein) which may be crucially involved in the Schiff base formation and Amadori rearrangement by bringing into spatial juxtaposition of carefully designed helical peptides, is a noteworthy step in the mechanistic understanding of protein glycation, with particular reference to the catalysis of Amadori rearrangement involved in the process (Venkatraman, 2001).

2.5.1.2 Methodologies to estimate HbA1c

There remain numerous analytical problems associated with glycated hemoglobin measurement, such as the lack of assay standardization and the problems related to its measurement in particular patient groups with haemoglobinopathies, fetal haemoglobin, renal failure and haemolytic diseases.

Methods of HbA1c assays have primarily evolved around three basic methodologies:

- (1) Based on difference in ionic charge.
- (2) Based on structural characteristics.
- (3) Based on chemical reactivity.

2.5.1.3 Methods based on differences in ionic charge

These methods are in extensive use at present. Cation exchange chromatography can either be undertaken on mini columns or in a sophisticated, automated system. The pH and temperature conditions affect the results significantly, hence, the need for a sophisticated system where the conditions can be adequately controlled. HbA1c possesses less charge positivity and hence elutes faster from a cation exchange column. Pre-glycohaemoglobin has similar mobility in this system and hence, it should be removed before column chromatography. Most of these systems cannot differentiate between abnormal haemoglobins, but many advanced systems possess such ability. These methods are most commonly used in clinical practice. By this method HbA1c concentration in normal subjects is 4.6–6%. Diabetes is considered to be under good, fair or poor control at values of < 7%, 7–8%, and > 8% respectively. HbA1c can be separated from HbAo by any electrophoretic method. The most commonly used method is agar gel electrophoresis where HbA1 migrates to cathodic side of HbAo. Pre-GHb migrates with GHb in this system as well and hence has to be separated in advance. Some haemoglobinopathies, like HbS or HbC trait do not, but HbF does interfere with this method.

2.5.1.4 Methods based on structural characteristics of HbA1c

One such method utilizes a column containing *m*-aminophenylboronic acid coupled to agarose. GHb possesses more *cis*-diol groups, which has stronger affinity to boronic acid and hence elutes later than HbAo. This method is

influenced to a lesser extent by pH, temperature and storage conditions than cation exchange chromatography. It is also unaffected by haemoglobinopathies. However, there is a batch-to-batch variation in gel characteristics, which makes application of this method difficult (**Fluckiger and Winterhalter, 1976**). Recently, immunoassays have been developed by using an HbA1c specific monoclonal antibody. An agglutinator is used in the system and inhibition of agglutination of HbA1c and its antibody by HbA1c in the sample is quantitated. This method is not influenced by pre-GHb or haemoglobinopathies (**Fluckiger and Winterhalter, 1976**).

2.5.1.5 Methods based on chemical reactivity

Chemical method of HbA1c estimation is based on generation of 5-hydroxymethylfurfural (5HMF) from glycoamino groups on haemoglobin, by heating the HbA1c in a weak acid. This chemical method measures total glycated hemoglobin, i.e. HbA1c plus glycated non-N terminal sites. The 5HMF so generated is reacted with thiobarbituric acid and read colour calorimetrically. This method is laborious but least expensive (**Iyer and Krishnaswamy, 1980**). It is a very sturdy method; least affected by storage, temperature and pH conditions. It is not influenced by pre HbA1c, but it is advisable to remove free glucose from samples by careful wash of erythrocytes before undertaking lysis. This method is not affected by haemoglobinopathies. Instead of an HbA1c standard, which is difficult to device, a fructose standard can be used and results expressed as amount of 5HMF. As this method estimates HbA1c as well as HbA1a and HbA1b, the

values obtained are higher than those of anion exchange chromatography by 1–2%. By their method, good, fair and poor control is defined as GHb level of < 8%, 8–10% and > 10% respectively.

2.5.1.6 Targets for patients with diabetes

The publication of the Diabetes Control and Complications Trial Research Group (DCCT, 1993) and U.K. Prospective Diabetes Study (UKPDS, 1998) has led to a reappraisal of the glycaemic control targets that should be aimed for in the treatment of patients with type 1 and type 2 diabetes. Previously, some guidelines tried to account for the lack of standardization in glycated haemoglobin measurement by comparing patients using the number of standard deviations (SDs), their HbA1c results from their particular assay's non-diabetic mean value. However, the SD targets were necessarily rather arbitrary and the use of SDs could lead to discrepancies in patient classification, depending on which glycated haemoglobin assay was used (Kilpatrick *et al*, 2000). The DCCT and the UKPDS have allowed a more 'evidence based' approach to be taken to the recommendations, and the fact that they both used the same HbA1c method has allowed SD targets to be dispensed with. The European diabetes policy group guidelines (as part of the International Diabetes Federation) now recommend that both patients with type 1 and type 2 diabetes aim for a DCCT or DCCT equivalent assay value of < 7.5% to reduce the risk of microvascular complications.

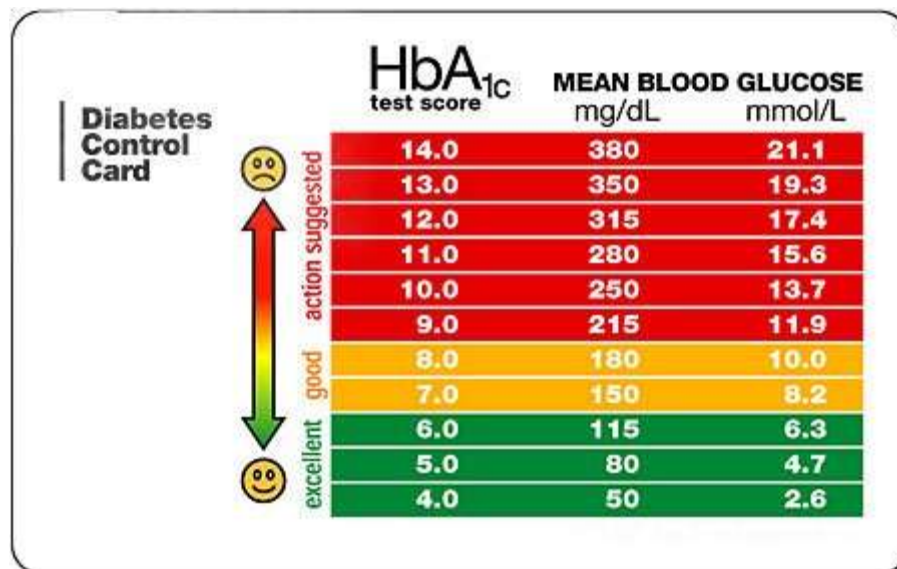


Figure 2-2: Chart showing HbA_{1c} and Mean Blood Glucose Comparison (Source: Embee Diagnostics, 1996).

2.5.1.7 HBA_{1c} as a screening test for diabetes

There remains considerable interest in extending the use of glycated haemoglobin measurement to include the diagnosis besides the monitoring of diabetes. Most studies have brought out the fact that glucose tolerance test (GTT) is a more sensitive diagnostic method and impaired GTT may occur with normal HbA_{1c}. Using the 1985 WHO oral glucose tolerance test (OGTT) criteria for diagnosing diabetes a meta-analysis of 34 studies has found HbA_{1c} to be of limited value as a screening test because of the large number of subjects who have either impaired glucose tolerance or frank diabetes, but have HbA_{1c} values that are within the

non-diabetic reference interval. Thus, a raised HbA1c would appear to be specific for diagnosing diabetes, but the test is not particularly sensitive (**WHO, 1985**). Many authors have suggested postprandial blood glucose along with HbA1c to improve the sensitivity in diagnosis of diabetes.

Even when using the proposed new diabetes (**Alberti and Zimmet, 1998**) which define diabetes as a fasting plasma glucose value ≥ 7 mmol/l, the same limitation in diagnosing type 2 diabetes by using GHb (HbA1c) is found (**WHO, 1997**). Some authors support the idea that HbA1c testing is likely to be a more physiological assessment of glucose intolerance than the artificial conditions of the OGTT, and so believe this should be the preferred diagnostic test. Certain studies have shown HbA1c to be as good a predictor of microvascular disease as fasting or two-hour post-OGTT glucose values, although not all studies have reached this conclusion (**Liu *et al*, 1993**). In addition to the difficulties arising out of methodologies used in estimating HbA1c, a few additional clinical considerations may influence the HbA1c values. It has been demonstrated that HbA1c can be high with normal GTT and high values can occur in non-diabetics (**Modan *et al*, 1988**). A genetic polymorphism has been described which influences the rate of glycation but probably the prevalence of such polymorphism is low. It is unlikely that HbA1c will ever be a reliable test for the diagnosis of type 2 diabetes for the following reason. If hyperglycaemia, rather than glycation, is the true cause of diabetic complications (and it continues to be the means of diagnosing diabetes) then HbA1c is fundamentally limited by the fact that two individuals with the same

degree of glucose tolerance can have HbA1c values that differ by nearly 2%. Thus, a subject with an HbA1c value of 4% would need to increase his/her glycation rate by 50% to match another non-diabetic subject with an HbA1c value of 6%. It is therefore not surprising that there can be overlap between the HbA1c values of patients with diabetes and those of subjects without the disease. Even if glycation is thought to be the underlying reason for complications, we have to be sure that glycation of haemoglobin gives an accurate reflection of glycation in small vessels. Because it is known that HbA1c values can be affected by factors that are independent of glycaemia or glycation rates this assumption may not be valid (Yudkin *et al*, 1996).

2.5.1.8 Frequency of HbA1c testing

There is controversy on the optimal frequency of GHb testing (Goldstein, *et al*, 2004). In the absence of evidence-based guidelines, expert opinion recommends HbA1c testing at least twice a year in patients who have stable glycaemic control and more frequently (quarterly assessment) in those whose therapy has changed or who are not meeting glycaemic goals (McIntosh *et al*, 2001). On the other hand, National Commission for Quality Assurance do not recommend the use of HbA1c more than two times a year (National Committee for Quality Assurance USA, 1999). There are also other reports suggesting more frequent monitoring (every 4 or 8 weeks) of patients with major change in therapy and of diabetic pregnancy. Considering the relatively high prevalence of diabetes inappropriate use of HbA1c

test frequent repetition of HbA1c test can be expected. Redundant laboratory tests are an important component of repeat laboratory testing (**Valenstein *et al*, 1988**). The appropriateness of laboratory utilization is especially important for a developing country with limited financial resources.

2.5.1.9 Importance of HBA1c

Glycated haemoglobin is a measure of blood glucose levels over the previous 120 days and reflects the time averaged blood glucose over the preceding 1–3 months, depending on the rate of change of blood glucose levels. HbA1c, or glycosylated haemoglobin, is formed through the non-enzymatic binding of circulating glucose to haemoglobin (glycation). HbA1c is measured as the ratio of glycosylated to non glycosylated haemoglobin (**Sacks *et al*, 2002**). Higher levels of glucose in the blood contribute to more binding and consequent higher levels of glycosylated haemoglobin. Glycation occurs over the entire 90-120 day life span of the red blood cell. HbA1c can consequently be interpreted as an average of the blood glucose present over the past 3-4 months. Measurement of HbA1c is accepted as a useful index of mean blood glucose in the treatment of patients with diabetes (**Sacks *et al*, 2002**). Decisions regarding treatment are often based on HbA1c. Although the **American Diabetes Association 2003** does not currently recommend HbA1c measurement for the diagnosis of diabetes, studies have shown frequency distributions similar to those of fasting plasma glucose (FPG) used in diagnosing diabetes, HbA1c is a more comprehensive measure of total glycaemic exposure than FPG due to the representation of blood glucose in the postprandial state in

addition to the fasting state. High HbA1c concentration is associated with diabetic microvascular complications, macrovascular complications, risk of death and cardiovascular disease (McIntosh *et al*, 2001). The American Diabetes Association 2003 recommended treatment goal for the diabetic population is HbA1c < 7%, although the target may be higher for older adults HbA1c levels as low as 6.2% have been cited as at risk threshold values for development of cardiovascular complications. Clinical and population studies have found racial, ethnic, and age disparities in HbA1c levels.

2.5.1.10 Factors affecting HbA1c

The National Committee for Quality Assurance (1999) states that HbA1c test is useful for determining blood glucose averages for the previous two or three months. Patients do not need to be fasting when blood is drawn, but several other factors can affect the test.

2.5.1.11 Blood Sugar

A normal HbA1c averages 6 percent or less and an average of 6.5 percent or more indicates diabetes. Hyperglycaemia, or high blood sugar, increases the percentage of glycosylated haemoglobin. The reverse is also true; hypoglycaemic episodes will reduce HbA1c readings but may not reflect proper management of diabetes. Normal or low HbA1c levels in a diabetic patient may indicate the use of too much insulin (Mokdad *et al*, 2000).

2.5.1.12 Red cell survival

Any situation which shortens erythrocyte survival or decreases mean erythrocyte age falsely lowers HbA1c test results regardless of the assay method (**Panzer *et al*, 1982**) showed that levels of HbA1c were significantly lower in people with haemolytic anaemia compared with people with non-haemolytic anaemia and normal controls. They demonstrated a curvilinear correlation between HbA1c and red cell survival.

2.5.1.13 Iron deficiency anaemia

Iron-deficiency anaemia increases HbA1c (**Tarim *et al*, 1999**). Among people with type 1 diabetes, iron deficiency anaemia is associated with higher concentrations of HbA1c and iron replacement therapy leads to a drop in HbA1c in both patients with and without diabetes.

2.5.1.14 Blood transfusion

Blood transfusions which includes RBCs from a person who does not have diabetes will reduce the average level of glycation of circulating haemoglobin, and hence reduce the HbA1c (**Panzer *et al*, 1982**) and it can take 1–2 months before HbA1c level is restored to a level reflecting blood glucose control. However transfusions can also increase HbA1c.

2.5.1.15 Haemoglobin variants

Bry *et al* (2001) performed a systematic review on the effects of haemoglobin variants and chemically modified derivatives on glycated haemoglobin assay

methods. Genetic variants and chemically modified derivatives of haemoglobin can have profound effects on the accuracy of HbA1c measurement, but these effects vary considerably with the different commercially available methods. Commonly encountered haemoglobin variants include HbS, HbC, HbE, and HbF and these variants are not uncommon in people with diabetes (**Bry *et al.*, 2001**). Differing effects of common and uncommon haemoglobin variants illustrate the need for laboratories to report the effects for their particular assay and for health professionals to be aware of the potential effect. In general, affinity chromatography methods show no interference from any of these haemoglobin variants and derivatives and are the assay method of choice for people with haemoglobin variants. Abnormal haemoglobin variants interfere mainly with cation exchange methods in either a positive or negative way depending on the individual manufacturers' separation system. The effect is less on immunological methods compared with many cation exchange methods but both positive and negative effects have been reported (**Bry *et al.*, 2001**).

2.5.1.16 Uraemia

Urea spontaneously dissociates in vivo to form ammonia and cyanate and the latter form is cyanic acid which can react with the N-terminal valine of the haemoglobin β chain to form carbamylated haemoglobin. One mmol/L urea is associated with the formation of 0.063% carbamylated haemoglobin and some uraemic patients may have carbamylated HbA1c as high as 3% of total HbA1c. Carbamylated haemoglobin interferes with HbA1c assayed by HPLC and

electrophoresis but not by affinity chromatography and immunoassay (**Weykamp *et al.*, 1993**).

2.5.1.17 Vitamins C and E

Vitamin C can falsely lower HbA1c results, possibly by inhibiting glycation of haemoglobin. **Davie *et al* (1992)** studied 12 subjects without diabetes who consumed 1 g/day vitamin C for 3 months. Although there were no significant changes in fasting glucose, HbA1c measured by affinity chromatography decreased 18%, from 6.2% at the start to 5.1% ($p < 0.0001$) after 3 months, whereas, HbA1c measured by electrophoresis increased 16%, from 6.2% to 7.2% ($p < 0.0001$) over the 3 months. **Ceriello *et al* (1991)** studied the effects of daily vitamin E supplementation of 600 mg and 1200 mg for 2 months in people with insulin-requiring diabetes and demonstrated reduced protein glycosylation independent of changes in plasma glucose, an effect that may be due to the inhibition of labile glycosylation.

2.5.1.18 Hypertriglyceridaemia

Falko *et al* (1982) reported in a person with diabetes that marked hypertriglyceridaemia resulted in a significant false increase in HbA1c measured by the cation-exchange chromatographic method. Conversely **Garrib *et al* (2003)** reported falsely low HbA1c in a person with diabetes and hypertriglyceridaemia measured by affinity chromatography but the result was unaffected when measured by HPLC and immunoturbidimetric methods.

2.5.1.19 Aspirin

Acetylated haemoglobin can interfere with HbA1c measured by HPLC and electrophoresis but not by affinity chromatography and immunoassay (**Weykamp *et al.*, 1993**). However levels of acetylated haemoglobin formed from chronic use of small doses of acetyl salicylate (200–300 mg/day) or brief use of higher doses (2000 mg/day for one week) are not sufficient to interfere with HbA1c measurements.

2.5.1.20 Race

Blacks tend to have higher levels of HbA1c than whites, (**Garber *et al.*, 2010**). None of the subjects (blacks or whites persons) were diabetic. Haemoglobin A1c levels were found to be higher in blacks than whites, regardless of normal blood glucose levels. The results indicate that the value of HbA1c in screening for diabetes, assessing the efficacy of treatment or predicting the likelihood of complications may be limited (**Garber *et al.*, 2010**).

Relaxation

Psychiatrists at the **Medical University of Ohio (2009)** conducted a randomized controlled trial to determine the effects of biofeedback-assisted relaxation on HbA1c in those with type 2 diabetes. Significant decreases in blood sugar, HbA1c, anxiety and depression were associated with biofeedback-assisted relaxation.

2.5.1.21 Glycated serum proteins

Measurement of glycation of serum proteins is another option for assessing longer term glycaemic control. Since human serum albumin has a half-life of

approximately 14 days, the degree of glycation of albumin provides an index of glycaemia over a shorter period of time than glycated haemoglobin (**Windeler and Kobberling, 1990**). The term fructosamine was originally introduced as a general term for glycated protein. However the term is now used to refer to the specific analyte measured by the nitroblue tetrazolium (NBT) assay, which is known as the fructosamine assay (**Goldstein *et al.*, 2004**). Measurements of total glycated serum proteins and glycated serum albumin have been suggested as alternative methods for routine monitoring of glycaemia in people with diabetes, however, there remain a number of unresolved problems with the assay. Glycated proteins are an alternate measure of blood glucose control but there are no data on their relationship with chronic diabetes complications. The relationship between fructosamine and HbA1c results has been assessed in a number of studies. In people with diabetes there is also a good correlation between the HbA1c and fructosamine. **Kruseman *et al.*, (1992)** indicate that although fructosamine and HbA1c are correlated, there are important differences between the two methods in assessing blood glucose control. HbA1c is considered the benchmark measure because all outcome studies of blood glucose control and diabetes complications have used this measure and there are no data on diabetes outcomes and blood glucose control assessed by fructosamine or other glycated proteins.

2.5.1.22 Self monitoring of blood glucose (SMBG)

Many aspects of SMBG were reviewed at a consensus meeting (**Bergenstal and Gavin, 2005**). In general SMBG adds information which complements glycated

haemoglobin data by providing real-time feedback to people with diabetes, their carers and health professionals. It allows detection of hypoglycaemia and hyperglycaemia which can improve safety and also helps to motivate people with diabetes to make appropriate treatment changes. In addition to evaluating blood glucose control, SMBG is an educational tool to inform both patient and health care professionals about the effects of lifestyle, behavioural and/or medication changes and to be fully effective requires ongoing education and reinforcement about the use of the data.

Numerous studies have been performed since the introduction of SMBG in the late 1980s demonstrating that available meters are sufficiently accurate for this purpose examined. SMBG is common among people with diabetes in developed countries; however its use is influenced by several factors (**Chen *et al*, 2003**). Despite use of SMBG for over 25 years, a number of issues remain unresolved, including the frequency and timing of testing, whether SMBG per say is associated with improved diabetes outcomes, and which category of people with diabetes should perform SMBG. Examining a possible relationship between blood glucose control and frequency of SMBG is difficult because of the interdependency of factors which influence blood glucose control and the limitations of cross-sectional studies. For example, SMBG is more likely to be recommended in people with poorer glycaemic control.

Hoffmann *et al*, (2008) evaluated once- and twice-daily SMBG testing strategies compared with four-times daily testing in assessing glycaemic control and

detecting hypoglycaemia or hyperglycaemia in people with stable insulin-treated type 2 diabetes. It was found out that the latter had better control than the once- and twice daily SMBG testing strategy.

2.5.1.23 Urine testing

Semi-quantitative pre-prandial urine testing to assess blood glucose control has major limitations, particularly in people with type 1 diabetes, but also in those with type 2 diabetes where a raised renal threshold for glucose may mask persistent hyperglycaemia. Negative urine tests fail to distinguish between normal and low blood glucose levels, which is a particular disadvantage since the aim of treatment is a normal blood glucose level while avoiding hypoglycaemia. However, urine glucose testing with visually read strips is still used by many people with type 2 diabetes and is satisfactory in those treated with diet alone or in those taking oral therapy that has stable glycaemic control (**Frier and Fisher, 1999**).

Chapter 3

MATERIALS AND METHODS

3.1 AREA OF STUDY STATISTICS

Ethical clearance was sought before commencement of the project from the committee on Human Research, Publication and Ethics (CHRPE), School of Medical Sciences, KNUST/KATH. Type of Study-Cohort retrospective. This study was approved by the Committee on Human Research, Publication and Ethics (CHRPE), School of Medical Sciences, KNUST/KATH. A written informed consent was taken from the subjects.

This work was carried out at the Department of Molecular Medicine, School of Medical Sciences, KNUST and the Diabetes and Hypertensive Clinic, Dormaa Presbyterian Hospital, Dormaa Ahenkro, Brong Ahafo Region under the supervision of Dr E. F. Laing from August 2010 over a period of two years. The hospital is a mission health facility and also serves as the only municipal hospital. It is one of the four Presbyterian Church of Ghana hospitals in the country. It was founded in 1955 and upgraded to a district hospital in 1978. The district has an estimated population of 180000 (Population and Housing census, 2000) with an annual growth rate of about 2.1%. Other clients from the neighbouring La Cote d'Ivoire and Sefwi area in the Western region enjoy health care from the facility. The hospital serves clients within a catchment area of approximately 3375 km².

The hospital provides general services in surgery, paediatrics, internal medicine, obstetrics, gynaecology and ophthalmology. It has 200 bed capacity with an

occupancy rate of 73 % (2010). The laboratory is furnished with the state of the art equipment which conducts investigations like haematology, microbiology, blood transfusion and biochemistry.

3.2 STUDY DESIGN

The present study had 2 groups of subjects, normal and diabetic subjects. Two categories of subjects were strictly selected according to WHO criteria and examined under this study; these groups were Established Diabetics on treatments (DM-T) with a sample size of 100 and Non Diabetic individuals (NG) with a sample size of 50. All diabetic subjects were already receiving routine therapy at entry into the study, but we made a renewed attempt to achieve optimum control on blood glucose by the use of glycated haemoglobin levels. Parameters recorded under this study included the following: Age, Height, Weight, Body Mass Index (BMI), Duration of disease, Haemoglobin (Hb), Fasting Plasma Glucose, (FPG) HbA1c (Glycated Haemoglobin).

3.3 STATISTICAL METHODS

The results were expressed as Mean $\pm$ SEM (Standard Error of the mean) instead of Standard Deviation which compares population. Unpaired - t- test was used to compare mean variables of diabetics and non diabetics. Statistical significant level was put at $P < 0.05$ unless otherwise stated. GraphPad Prism version 5.00 for windows was used for statistical analysis (GraphPad software, San Diego California USA, www.graphpad.com). Comparison between the investigated

anthropometric, clinical and laboratory parameters were established using Pearson's correlation coefficient (r).

Bland-Altman method using 95% confidence intervals (95% CIs) from the multivariate logistic regression models were used to compare HbA1c among different glycaemic levels of diabetics and nondiabetics. Receiver operative characteristics (ROC) curve was used to discriminate between glycaemic levels using HbA1c test. Regression graphs were plotted between HbA1c and blood glucose to determine the goodness of fit using coefficient of regression. Correlation was significant at the 0.05, 0.01, 0.001 levels (2 tailed).

3.4 SUBJECT SELECTION

3.4.1 Inclusion Criteria

The study used different criteria to identify and recruit the different categories of subjects ranging between the ages of 21 and 86 years. Non diabetic individual subjects were recruited from the general adult population from the Dormaa Municipal Area using fasting plasma glucose. Naturally formed groups like health professionals and churches were screened and identified. The criteria used was FPG below 6.0 mmol/L. Out of the 100 normoglycaemic individuals 50 subjects were selected randomly to participate in the study. The control samples were treated similarly as the test samples.

Established DM patients on treatment (DM-t) were purposely selected from patients attending the diabetes and hypertensive clinic at the Dormaa Presbyterian

Hospital from their medical records where their last three to six months FPG values were retrospectively grouped into the following category for the purpose of this study as consistently low (Hypoglycaemia $<3.5\text{mmol/l}$, 20), consistently (normal $3.5\text{--}5.9\text{ mmol/l}$, 20, consistently above normal $6.0\text{--}6.9\text{ mmol/l}$, 30 and hyperglycaemia (≥ 7.0) mmol/l , 30. The low normal and high normal categories were generalized as Normoglycaemia. The study involved the measurement of Anthropometric, Clinical and Laboratory variables.

The anthropometric variables being age (years), duration of disease (six months to five years), weight (kilogram) and height (metres), and computed body mass index (kg/m^2). BMI was calculated as weight $\text{kg}/\text{height squared}$ (kg/m^2) and subjects were considered as normal weight if their BMI was $< 25\text{ kg/m}^2$, overweight if their BMI was from 25 to 29 kg/m^2 and obese if their BMI was $\geq 30\text{ kg/m}^2$. The clinical variables include systolic blood pressure and diastolic blood pressure (mmHg). Those whose average systolic blood pressure (SBP) was $\geq 140\text{ mmHg}$ and/or diastolic blood pressure (DBP) $\geq 90\text{ mmHg}$ were defined as hypertensive.

The laboratory parameters included haemoglobin concentration Hb, grams/deciliter), instant fasting plasma glucose (FPG, mmol/l) and glycosylated haemoglobin (%HbA1c). The principal parameter for the study was however HbA1c. All anthropometric assessments were recorded by qualified nurses using standardized equipment such as weighing scales (for weight measurement to the nearest 0.1kg in light clothing on a bathroom scale) and stadiometer (for measurement of height to the nearest centimeter without shoes against wall

mounted ruler) (Zhongshan Camry Electronics Limited, Guangdong, China). Selected subjects were interviewed for their ages and measurements of their heights and weights were taken. Their BMIs were computed from their weight and height. Blood pressure diastolic and systolic measurements were done using a sphygmomanometer apparatus (3M Health Care, St Paul, MN 55144, 1000 USA) from the left upper arm by qualified nurses.

The study involved a general comparative study of how well the HbA1c levels correlate with instant fasting plasma glucose concentration and mean fasting plasma glucose as a potential tool for monitoring glucose control in diabetes patients over a three month period.

3.4.2 Exclusion criteria

Diabetics and control subjects with renal or liver diseases or any other serious illness were excluded. Also subjects with haemoglobinopathies were excluded from the study. Subjects taking medications such as aspirin, Vitamin C and E were also dropped. These were verified by first checking from their medical records and through the use of a questionnaire.

3.5 SAMPLE COLLECTION

The sample collection for the study was done in two stages. Using patients' medical records 100 known DM patients were systematically selected without any collection of participants' blood. Stage two - the second stage of the study involved

blood specimen estimation for fasting blood glucose for the screening of non-diabetic individuals.

Blood samples (10ml) were obtained from all the participants and their HbA1c levels were estimated using the DCA 2000+ analyzer (Bayer Corporation, USA) while Hb levels were measured using semi - automated haematology analyzer.

3.5.1 Materials for fasting plasma glucose estimation

The following materials and reagents were obtained from Elan diagnostics, Thurber Boulevard, Smithfield R102917, USA.

A. ATAC 8000 Random Access Chemistry analyzer

B .Reagents;

1. Reagent ATAC PAK Product No 532 – 008, 8 X150 Tests.

2. ATAC Calibrator

3. ATAC Normal and Abnormal control serum

4. Split boat caps

5. Glucose reagent boats

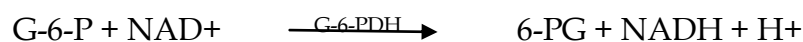
6 .Glucose activator bottles

Samples were stored in a refrigerator (Robert Mc Bride Ltd, Middleton, UK) at 2- 8 °C.

3.5.1.1 Methodology: Blood Glucose Estimation

All the blood specimens for fasting plasma glucose were taken after subjects have fasted overnight. Two milliliters (2ml) of fasting venous blood samples were collected in sodium fluoride vacutainers under aseptic precautions from subjects. Age, gender and duration of diabetes were noted. The blood was analyzed for glucose. The JAS diagnostics glucose hexokinase method is based on a modification of the Slein Enzymatic method which uses hexokinase and glucose-6-phosphate dehydrogenase to catalyze the reaction.

Hexokinase reaction principle



Glucose was phosphorylated with adenosine triphosphate (ATP) in the reaction catalysed by hexokinase (HK). The product glucose- 6-phosphate was oxidized with the concomitant reduction of nicotinamide adenine dinucleotide (NAD) to reduced nicotinamide adenine dinucleotide (NADH) in the reaction catalysed by glucose- 6- phosphate dehydrogenase (G-6-PDH). The formation of NADH caused an increase in the absorbance at 340 nm. The increase was directly proportional to the amount of glucose in the sample.

3.5.1.1 Precautions In Glucose Assay

The use of prescribed sample tubes were adhered to in order to preserve the glucose content of the specimen- sodium and potassium salts of fluoride and, oxalate. All specimens were inspected for the presence of significant levels of haemolysis and icterism and all such specimens were rejected. Turbid samples were rejected as well. Recalibration was done every time after loading of new reagents.

3.5.1.2 Reagent preparation

The working reagent was prepared by following manufacturer's instructions (**ATAC Operators manual, 2007**). One end of the plastic coupler was placed into the substrate bottle. The opposite end of the coupler was inserted into the diluent's bottle, inverted repeatedly until the reagent dissolved.

3.5.1.3 Reagent storage

- 1 Reagents were stored at room temperature
- 2 Combined working reagents was stable
- 3 Standards were stored at 2- 8 °C.

3.5.1.4 Storage and stability

Reconstituted reagent was stable for 30 days refrigerated at 2 – 8 °C. Open on board stability was two weeks.

3.5.2 Methodology For Glycated Haemoglobin (HbA1c) Estimation.

3.5.2.1 Reagents

The following materials and reagents were obtained from Bayer Corporation, Elkhart USA

1. DCA 2000 HbA1c analyser
2. Antibody Latex: HbA1c-specific mouse monoclonal antibody adsorbed onto latex particles. 2.5% w/v antibody-latex in 10mM glycine buffer; 16% w/v non-reactive ingredient (10uL dried in each reagent cartridge).
3. Agglutinator: 0.005% w/v poly (aspartic acid) polymer covalently attached to the HbA1c hapten in 20mM sodium citrate buffer containing 0.1% w/v bovine serum albumin and 1% w/v non-reactive ingredients (10ul dried in each cartridge).
4. Buffer Solution: 8.1% w/v lithium thiocyanate, 0.01% digitonin in 200mM glycine buffer (0.6 mL in each cartridge)
5. Oxidant: 1.5% w/v potassium ferricyanide in water mixed with 21% w/v non-reactive ingredients (100mL dried in each cartridge).

The DCA 2000 (Bayer Corporation, Elkhart, USA) HbA1c assay was chosen from among a number of methods available because of its high precision and convenience. It provided a convenient quantitative method for measuring the percentage concentration of HbA1c. Both the specific HbA1c and the total

haemoglobin were measured, and the ratio calculated as percent HbA1c. For the measurement of total haemoglobin, potassium ferricyanide was used to oxidize haemoglobin in the sample to methaemoglobin. The methaemoglobin then complexed with thiocyanate to form thiocyan- methaemoglobin, the coloured species which was measured. The extent of colour development at 531nm was proportional to the concentration of total haemoglobin in the sample.

3.5.2.2 Principle Of The Measurement Of HbA1c

An agglutinator (synthetic polymer containing multiple copies of the immunoreactive portion of HbA1c) caused the agglutination of latex coated with HbA1c specific mouse monoclonal antibody. This agglutination reaction caused increased scattering of light which was measured as an increase in absorbance at 531nm. HbA1c in whole blood specimen competes for the limited number of antibody-latex binding sites causing an inhibition of agglutination and decreased scattering of light. The decreased scattering was measured as a decrease in absorbance at 531nm. The HbA1c concentration is then quantified using a calibration curve of absorbance versus HbA1c concentration.

CALCULATIONS

The percent HbA1c in the sample is then calculated as follows

$$\% HbA1c = \frac{[HbA1c]}{[Total Haemoglobin]} \times 100$$

All measurements and calculations were performed automatically by the DCA 2000 + Analyzer (Bayer Corporation, Elkhart, USA) and the screen displayed percent HbA1c at the end of the assay.

3.5.2.3 Procedure For Glycated Haemoglobin Measurement

The power was turned on to warm the machine for about 1-2 minutes. The system was then calibrated for each new lot number of reagent cartridges by using a calibration card provided in the DCA 2000 haemoglobin A1c reagents kit. The foil package containing the reagent cartridge was opened. In filling glass capillary with blood obtained by venipuncture, the blood sample was mixed by inversion to prevent separation of red blood cells and plasma. The stopper was removed from the collection tube in such a way that a small sample of blood remained on the stopper. The capillary holder was then held at an angle so that it touched only the tip of the capillary to blood sample on the stopper. A lint free tissue was used to carefully wipe the outside of the glass capillary. The capillary holder was positioned in the correct orientation for insertion into the reagent cartridge. The capillary holder was inserted into the reagent cartridge until the holder gently snapped into place. In analysing the patient sample, the dot (on the instrument) next to the bar code track was located. The reagent cartridge was then inserted into the bar code track quickly (within 1 second) and smoothly slid down past the dot. The cartridge compartment door was opened and the reagent cartridge was inserted into it until subtle snap was heard.

Using a smooth, slow, continuous motion, the pull flexible plastic pull tab was completely out of the reagent cartridge. The door was closed and the flexible plastic pull tap was disposed off, five seconds after there was a beep sound and the assay began. The displayed results were recorded after 6 minutes before removing the reagent cartridge. The range for the test was 2.5% - 14%. A less than sign (<) in the display indicated a concentration below the lower limit of the test reported as (< 2.5% HbA1c) and may indicate that the sample contained substantial amounts of foetal haemoglobin (HbF). A greater than sign (>) indicated a concentration above the upper limit of the test and was reported as (> 14% HbA1c).



Figure 3-1: Bayer DCA 2000 analyzer

The Bayer DCA 2000 Analyzer is for the determination of glycated haemoglobin for long term monitoring of diabetes.

This analyzer efficiently performs haemoglobin A1c in few minutes.

The analyzer has on its front panel: reagent cartridge compartment access door, reagent cartridge compartment, display unit, keys, bar code reader window and bar code track. On its rear unit are the power switch, filter holder, programme card connector, printer output and power cord connector. This Bayer DCA 2000 glycohemoglobin analyzer requires no reagent preparation, mixing, or handling. The sample collection capillary holder is a central part of unique reagent cartridge. Up to 16 results can be stored in the memory, making record keeping very convenient.

Other features include screen display of all instructions, calibration status, testing information and results (**Bayer Corporation, Elkhart, USA**).

3.5.3 Methodology for haemoglobin estimation

3.5.3.1 Materials

- The following materials and reagents were obtained from (**Vital Scientific, Dieren, The Netherlands**).
- Microtech 3000 spectrophotometer (**Vital Scientific, Dieren, The Netherlands**).

3.5.3.2 Reagents :

- Potassium hexacyanoferrate ($K_3Fe(CN)_6$) (Reagent 1) (**Vital Scientific, Dieren, The Netherlands**).
- Potassium cyanide (KCN) (Reagent 1) (**Vital Scientific Dieren, The Netherlands**).

- Potassium dihydrogen phosphate(KH_2PO_4) (Reagent 2) (Vital Scientific Dieren The Netherlands).

The Cyanmethaemoglobin assay was chosen from among a number of methods available because of its high precision and convenience. It provided a convenient quantitative method for measuring the concentration of haemoglobin. The principle for the measurement of total haemoglobin: Potassium ferricyanide was used to oxidize haemoglobin in the sample to methaemoglobin. The methaemoglobin then complexed with thiocyanate to form thiocyan-methaemoglobin, the coloured species which was measured. The extent of colour development at 540nm was proportional to the concentration of total haemoglobin in the sample.

3.5.3.3 Procedure For Haemoglobin Concentration Measurement

- Reagent: Elitech Product No 534 – 006, 7x 40 tests were obtained from Vital Scientific Dieren, The Netherlands.

Reagent Preparation: Equal volumes of Reagent 1 and Reagent 2 were measured and mixed well.

Storage: Reconstituted reagent was stable for 30 days refrigerated at 2- 8 °C. Open on board stability was two weeks.

The following reference ranges were used as normal values for male and female subjects in the study. Male: 13 – 18 g/dl, Female: 12 – 16 g/dl, (Cheesbrough, 2002).

Chapter 4

RESULTS

4.1 ANTHROPOMETRIC, CLINICAL AND LABORATORY CHARACTERISTICS OF THE ENTIRE STUDY GROUP (DIABETICS AND NON DIABETICS (CONTROLS) AS STRATIFIED BY FASTING PLASMA GLUCOSE (TABLE 4.1).

From this study, the mean age of the diabetics (49.28 ± 1.42), normal (50.20 ± 0.81) above normal (58.36 ± 0.64) hyperglycaemia (50.12 ± 2.30) were similar to the mean age of non diabetics (48.98 ± 2.92) with no significant differences ($p > 0.05$). However, the mean age of the hypoglycaemic group (35.30 ± 1.74) was significantly lower than the non diabetics ($p < 0.01$).

The mean body mass indices (BMI) of diabetics (25.45 ± 0.38), hypoglycaemic group (25.93 ± 0.88) high normal group (25.10 ± 0.74) were all similar ($p > 0.05$) as compared to the control non diabetics (24.94 ± 0.42). However, normal group had significant BMI ($p < 0.001$) as compared to the control group. Also higher mean value for hyperglycaemic range was realized as significant ($p < 0.05$) as compared to the non diabetics. From the table mean systolic blood pressure of diabetics and its systematic groupings were significantly higher ($p < 0.001$) as compared to the control group. However there were no significant difference between diastolic pressures of both diabetics and nondiabetics.

The mean fasting plasma glucose of diabetics (5.17 ± 0.19) was similar ($p > 0.05$) to that of controls (5.17 ± 0.079). The hypoglycaemic, normal, above normal and hyperglycaemic blood glucose ranges did not show significant difference as shown

in the table below. The mean haemoglobin values of all diabetic groups showed highly significant decreases as compared to nondiabetics (Table 4.1). Mean levels of HbA1c among diabetics were significantly higher as compared to the control group.

Table 4-1: Anthropometric, Clinical And Laboratory Characteristics Of Diabetic Subjects and Non Diabetic Controls Stratified By Fasting Plasma Glucose Levels

Parameter	Age	BMI	SBP	DBP	Duration	FPG (M1)	FPG (M2)	FPG (M3)	MFPG	HB	HbA1c
Diabetics	49.28±1.42	25.45±0.38	126.50±2.49***	76.92±1.37	6.10±0.10	5.03±0.19	5.16±0.19	5.34±0.19	5.17±0.19	11.76±0.20***	6.58±0.14***
HypoG	35.30±1.74**	25.93±0.88	113.50±3.18***	75.50±3.12	5.98±0.15	3.19±0.04	3.25±0.03	3.34±0.02	3.27±0.01	12.12±0.28***	5.86±0.26*
Normal	50.20±0.81	25.43±0.71***	130.00±4.65***	76.5±2.33	6.025±0.12	4.72±0.19	5.15±0.14	5.46±0.12	5.08±0.13	11.6±0.39***	6.57±0.26***
Above Normal	58.36±0.64	25.10±0.74	137.90±5.46***	79.29±2.66	6.33±0.15	6.43±0.07	6.41±0.06	6.67±0.06	6.51±0.05	12.04±0.49***	7.01±0.27***
HyperG	50.12±2.30	26.07±0.48*	125.1±3.31***	80±1.68	5.29±0.16	8.97±0.29	8.79±0.29	8.86±0.27	8.86±0.26	11.97±0.29***	8.67±0.25***
Non diabetics	48.98±2.92	24.94±0.42	111.8±1.93	77.8±1.59	-	-	-	-	5.17±0.079	13.96±0.21	5.36±0.08

Data is presented as means ± Standard error of the mean. BMI- Body mass index, SBP-Systolic blood pressure, DBP- Diastolic blood pressure, FPG(M1)- Fasting plasma glucose at the first month, FPG(M2)-Fasting plasma glucose at the second month, FPG(M3)Fasting plasma glucose at the third month ,MFPG-Mean fasting plasma glucose,HbA1c – Average glycosylated haemoglobin concentration, HypoG = diabetic hypoglycaemia, HyperG = diabetic hyperglycaemia..*P is significant at the 0.05 level (2-tailed), **P is significant at the 0.01 level (2-tailed) ***P is significant at the 0.001 level (2-tailed). The diabetic group was compared to the non diabetic group using unpaired t-test.

4.2 PEARSON PRODUCT CORRELATION BETWEEN ANTHROPOMETRIC, CLINICAL AND LABORATORY INDICES OF FEMALE AND MALE DIABETIC SUBJECTS

Among the female subjects group in this study, monthly fasting plasma glucose, mean fasting plasma glucose and glycated haemoglobin levels all showed a significant positive correlations with age and systolic blood pressure. Also fasting plasma glucose at month two, positively correlated significantly with fasting plasma glucose at month one. Fasting plasma glucose at month three showed positive significant correlation with fasting plasma glucose at month one and two. Further mean fasting plasma glucose of the overall three month period also gave a significant positive correlation with fasting plasma glucose at the first, second and third months fasting plasma glucose. The glycated haemoglobin gave significant positive correlations with first, second, third and mean fasting plasma glucose of the female diabetic group. Thus there were direct relationships between the investigated parameters. Anthropometric indices like height and body mass index gave direct relationships with weight (Table 4.2).

Among the male diabetic group, as the age increases their level of monthly fasting plasma, mean fasting plasma as well as glycated haemoglobin increases just as in the female group. Also instant fasting plasma glucose at month two gave a direct relationship with fasting plasma glucose at month one just as in the female group. Similarly significant positive correlations occurred for month three with month two and one. Mean fasting glucose also correlated significantly with all the three month period fasting plasma glucose. Glycated haemoglobin levels correlated positively with mean fasting plasma glucose as well as the various

monthly glucose (Table 4.2). Again height and body mass index gave direct relationships with weight. Diastolic pressure on the lower left hand side also showed a significant positive correlation with systolic blood pressure.

Table 4-2: Pearson Product Correlation Coefficient between Anthropometric Indices, Clinical Variables and Laboratory Measurements: Female Diabetics (Upper Right- Hand Side) and Male Diabetics (Lower Left-Hand Side)

Parameter	Age	Weight	Height	BMI	SBP	DBP	Duration	FPG (M1)	FPG (M2)	FPG (M3)	MFPG	HB	HbA1c
AGE		-0.03	0.03	-0.07	0.44	0.05	0.12	0.87***	0.88***	0.88***	0.89***	-0.17	0.54***
Weight	0.09		0.71***	0.77***	-0.09	-0.04	-0.2	-0.18	-0.15	-0.12	-0.16	-0.01	-0.19
Height	-0.06	0.72***		0.11	-0.21	-0.06	-0.07	-0.10	-0.04	-0.02	-0.06	0.05	0.04
BMI	0.18	0.89***	0.32		0.05	-0.01	-0.24	-0.17	-0.19	-0.17	-0.18	-0.04	-0.29
SBP	0.44	0.25	0.29	0.15		0.30	-0.01	0.39**	0.41***	0.41***	0.41***	-0.09	0.30*
DBP	0.10	0.23	0.25	0.16	0.53***		-0.07	0.15	0.15	0.17	0.16	0.11	-0.04
Duration	0.01	-0.19	-0.11	-0.2	0.11	0.06		0.17	0.21	0.17	0.19	-0.09	0.07
FPG (M1)	0.61***	0.2	0.02	0.28	0.28	0.07	-0.09		0.94***	0.93***	0.98***	-0.15	0.52***
FPG (M2)	0.65***	-0.06	-0.25	0.08	0.35	0.03	0.2	0.66***		0.97***	0.99***	-0.19	0.49***
FPG (M3)	0.64***	0.05	-0.06	0.12	0.40	-0.05	-0.1	0.78***	0.78***		0.98***	-0.17	0.48***
MFPG	0.70***	0.07	-0.11	0.19	0.38	0.01	0.01	0.89***	0.89***	0.94***		-0.17	0.51***
HB	0.04	-0.14	-0.45	0.09	-0.22	-0.18	0.26	-0.03	0.19	-0.12	0.02		-0.1
HbA1c	0.41*	-0.05	-0.06	0.01	0.23	0.19	0.06	0.57***	0.55***	0.66***	0.65***	-0.22	

*Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed), ***Correlation is significant at the 0.001 level (2-tailed). BMI=body mass index, SBP=systolic blood pressure, DBP= diastolic blood pressure, FPG (M1) = fasting plasma glucose at first month, FPG(M2 = fasting plasma glucose at second month, FPG (M3) = fasting plasma glucose at third month, MFPG = mean fasting plasma glucose, HB = haemoglobin concentration, HbA1c = glycated haemoglobin.

4.3 CORRELATION BETWEEN HbA1c AND VARIOUS MONTHLY FASTING PLASMA GLUCOSE AS WELL AS THAT BETWEEN MEAN FASTING PLASMA GLUCOSE OF THE DIABETIC GROUP (TABLE 4.3).

The table below shows the correlation coefficient between HbA1c and monthly fasting plasma glucose as well as mean fasting plasma glucose. There were significant positive correlations between the various monthly instant fasting plasma parameters as well as the mean fasting plasma glucose. Also the correlation of HbA1c versus instant FPG for the first, second and third months respectively were calculated. The corresponding correlation coefficients were 0.750, 0.736, 0.757 respectively. Correlation coefficient between HbA1c and mean fasting plasma glucose gave 0.764 for the three month period. It was observed that the strongest positive linear correlation existed between the HbA1c concentration and the average FPG concentration for the three-month period.

Table 4-3: Pearson Product Correlation Coefficient between monthly fasting plasma glucose and glycated haemoglobin (HbA1c), as well as that between mean fasting plasma glucose.

	FPG (M1)	FPG (M2)	FPG (M3)	MFPG	HbA1c
FPG (M1)		0.918***	0.942***	0.975***	0.750***
FPG (M2)			0.948***	0.975***	0.736***
FPG (M3)				0.983***	0.757***
MFPG					0.764***

*Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed), ***Correlation is significant at the 0.001 level (2-tailed). HbA1c = glycated haemoglobin. BMI=body mass index, SBP=systolic blood pressure, DBP= diastolic blood pressure, Duration of diabetes, HB=haemoglobin levels.

4.4 CORRELATION ANALYSIS BETWEEN AVERAGE GLYCOSYLATED HAEMOGLOBIN AND ANTHROPOMETRIC AND CLINICAL VARIABLES FOR DIABETIC GROUP.

The table 4.4 showed correlation coefficient of the anthropometric and clinical variables with measured HbA1c for diabetic subjects. It was observed that a direct relationship exist between glycation and age of the study diabetic population with high coefficient of regression and a positive 95 percentage confidence interval. HbA1c however gave positive correlations with systolic, body mass index and diastolic blood pressures but they were insignificant. HbA1c also gave a negative significant correlation with duration of disease. HbA1c gave insignificant relationship with haemoglobin levels

4.4 CORRELATION COEFFICIENT WITH HbA1c

Table 4-4: Correlation of HbA1c versus anthropometric and clinical variables of diabetics

Parameter	Pearson r	95% CI	R square	P value
Age	0.3348	0.1481 to 0.4985	0.1121	0.0010***
BMI	0.0736	-0.1246 to 0.2663	0.0054	0.3955
SBP	0.1897	-0.0070 to 0.3723	0.0359	0.5609
DBP	0.1229	-0.0754 to 0.3118	0.0151	0.1798
Duration	-0.2837	-0.4548 to -0.0924	0.0804	0.0414**
Haemoglobin	-0.0572	-0.2509 to 0.1408	0.0032	0.2520

*Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed),***Correlation is significant at the 0.001 level (2-tailed). HbA1c = glycated haemoglobin. BMI=body mass index, SBP = systolic blood pressure, DBP= diastolic blood pressure, Duration of diabetes, HB = haemoglobin levels. Ns = non significant, CI = 95% confidence interval, R square = coefficient of regression.

4.5 CORRELATIONS BETWEEN HbA1c AND ANTHROPOMETRIC AND CLINICAL VARIABLES OF NON DIABETICS

From the table 4.6 below, HbA1c showed a significant negative correlation with age with positive goodness of fit, but gave positive non significant relation with fasting plasma glucose. HbA1c however gave negative non significant correlation with body mass index, systolic, diastolic blood pressures and haemoglobin levels.

4.6 FOR NON DIABETICS (CONTROLS)

Table 4-6: Correlation of HbA1c versus anthropometric and clinical variables of controls

Parameter	Pearson r	95% C I	P value	R square
Age	-0.359	-0.5795 to -0.0895	0.1176*	0.1289
BMI	-0.1112	-0.3779 to 0.1726	0.7053	0.0123
SBP	-0.2144	-0.4650 to 0.0681	0.3006	0.0459
DBP	-0.1338	-0.3974 to 0.1502	0.4500	0.0179
Haemoglobin	-0.2037	-0.4562 to 0.0792	0.4610	0.0414
FPG	0.1633	-0.1206 to 0.4225	0.6100	0.0266

*Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed),***Correlation is significant at the 0.001 level (2-tailed). HbA1c = glycated haemoglobin. BMI = body mass index, SBP = systolic blood pressure, DBP= diastolic blood pressure,HB = haemoglobin levels.FPG= fasting plasma glucose concentration. Ns = non significant,CI = 95% confidence interval and R square = coefficient of regression.

4.7 CORRELATION COEFFICIENT BETWEEN MEAN FASTING PLASMA GLUCOSE AND ANTHROPOMETRIC AND CLINICAL VARIABLES OF DIABETICS.

From the study table 4.7 below FPG showed a significant positive correlation with age, and systolic blood pressure. It however gave a negative significant relationship with duration of disease and haemoglobin. The rest all gave positive non-significant relationship with the abstracted FPG.

4.7 CORRELATION COEFFICIENT WITH FPG

Table 4.7: Correlation of Mean FPG against anthropometric and clinical variables of diabetics

Parameter	Pearson r	95% C I	P value	R square
BMI	0.0871	-0.1112 to 0.2788	0.1838	0.0075
SBP	0.2321	0.0373 to 0.4099	0.0061*	0.0538
DBP	0.1578	-0.0399 to 0.3436	0.3960	0.0248
Duration	-0.3474	-0.5091 to -0.1620	0.0037***	0.1207
Age	0.4857	0.3197 to 0.6227	0.0001***	0.2359
Haemoglobin	-0.3006	-0.4693 to -0.1106	0.002**	0.0903

*Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed), ***Correlation is significant at the 0.001 level (2-tailed). HbA1c = glycated haemoglobin. BMI = body mass index, SBP = systolic blood pressure, DBP= diastolic blood pressure, HB = haemoglobin levels. FPG= fasting plasma glucose concentration. Ns = non significant, CI = 95% confidence interval and R square = coefficient of regression.

4.8 BLANT-ALTMAN COMPARISON OF HBA1C AMONG GLYCAEMIC LEVELS IN DIABETICS AND NON DIABETICS

From the table 4.8 below Bland - Altman method was used to compare HbA1c among different glycaemic levels of diabetics and nondiabetics. The average HbA1c level of diabetics hypoglycaemia, normoglycaemia and hyperglycaemia were greater than that of non diabetics and thus deviated from non diabetics by bias of -0.35 ± 1.37 , -1.456 ± 1.246 , -3.339 ± 1.626 , 95% CI ($-3.045 - 2.355$, $-3.898 - 0.985$, $-6.526 - 0.152$) respectively. The average HbA1c of diabetic normoglycaemia was greater than that of diabetic hypoglycaemia and hypoglycaemia deviated from normoglycaemia by bias of -0.710 ± 1.800 CI ($-4.240 - 2.820$). The average HbA1c of diabetic hyperglycaemia was greater than diabetic normoglycaemia and deviated

from normoglycaemic by a bias of -1.779 ± 1.805 , CI $(-5.317 - 1.758)$. The average HbA1c of diabetic hyperglycaemia was also greater than diabetic hypoglycaemia and deviated from hypoglycaemia by a bias of -1.885 ± 1.221 , $(-4.278 - 0.508)$.

Table 4-8: Bland Altman comparison of HbA1C among different glycaemic levels

Parameter	Bias	95% CI	%Bias	95%CI
Non diabetics/DhypoG	-0.35 ± 1.37	$(-3.045 - 2.355)$	-4.370 ± 23.370	$(-50.01 - 41.44)$
Non diabetics/DnormoG	-1.456 ± 1.246	$(-3.898 - 0.985)$	-23.33 ± 20.890	$(-64.28 - 17.61)$
Non diabetics/DhyperG	-3.339 ± 1.626	$(-6.526 - 0.152)$	-46.39 ± 18.710	$(-83.07 - 9.707)$
DnormoG/DhypoG	-0.710 ± 1.800	$(-4.240 - 2.820)$	-11.52 ± 30.110	$(-70.53 - 47.49)$
DnormoG/DhyperG	-1.779 ± 1.805	$(-5.317 - 1.758)$	-22.63 ± 22.550	$(-66.82 - 21.56)$
DhypoG/DhyperG	-1.885 ± 1.221	$(-4.278 - 0.508)$	-28.87 ± 19.180	$(-66.47 - 8.735)$

Data is presented in figures and percentages. Dhypo = diabetic hypoglycaemia, DnormoG=diabetic normoglycaemia, DhyperG = diabetic hyperglycaemia. mBias, 95 % confidence interval,% Bias

4.9 RELATIVE SENSITIVITY AND SPECIFICITY OF HBA1C AMONG DIABETICS AND NON DIABETIC SUBJECTS (FIGURE 4.1)

In the graph below glycated haemoglobin (HbA1c test) was to draw the threshold between normal and abnormal blood glucose ranges. In doing this an ROC (Receiver operative characteristics) was used to draw the line between 'normal' and 'not normal' blood glucose. The area under the curve (AUC) was more than 0.5 to be considered as specific. In the first part of the figure graph was constructed between non diabetics versus different degrees of glycaemic levels.

The glycated haemoglobin levels between normal controls and diabetics with hypoglycaemia was not sensitive ($AUC = 0.628 \pm 0.086$, $P = 0.087$) meaning HbA1c could not detect individuals with these conditions.

HbA1c gave a significant difference between diabetics with normoglycaemia and non diabetics ($AUC = 0.911 \pm 0.039$, $P < 0.0001$). This means HbA1c can detect between individuals with controlled blood glucose and healthy people without diabetes. The HbA1c between diabetics with hyperglycaemia and normal control was highly significant. This means HbA1c can detect between these individuals ($AUC = 1.00 \pm 0.00$, $P < 0.0001$).

The second part of the graph showed relation between various degrees of diabetes using HbA1c test. The curve between diabetic hypoglycaemia and normoglycaemia was significant ($AUC = 0.739 \pm 0.075$, $P = 0.0028$). Diabetics with normoglycaemic and hyperglycaemic gave a significant curve using HbA1c test ($AUC = 0.860 \pm 0.040$, $P < 0.0001$). The difference between hypoglycaemic and hyperglycaemic was highly significant. This means that HbA1c is more sensitive towards hyperglycaemia ($AUC = 0.934 \pm 0.035$; $P < 0.0001$).

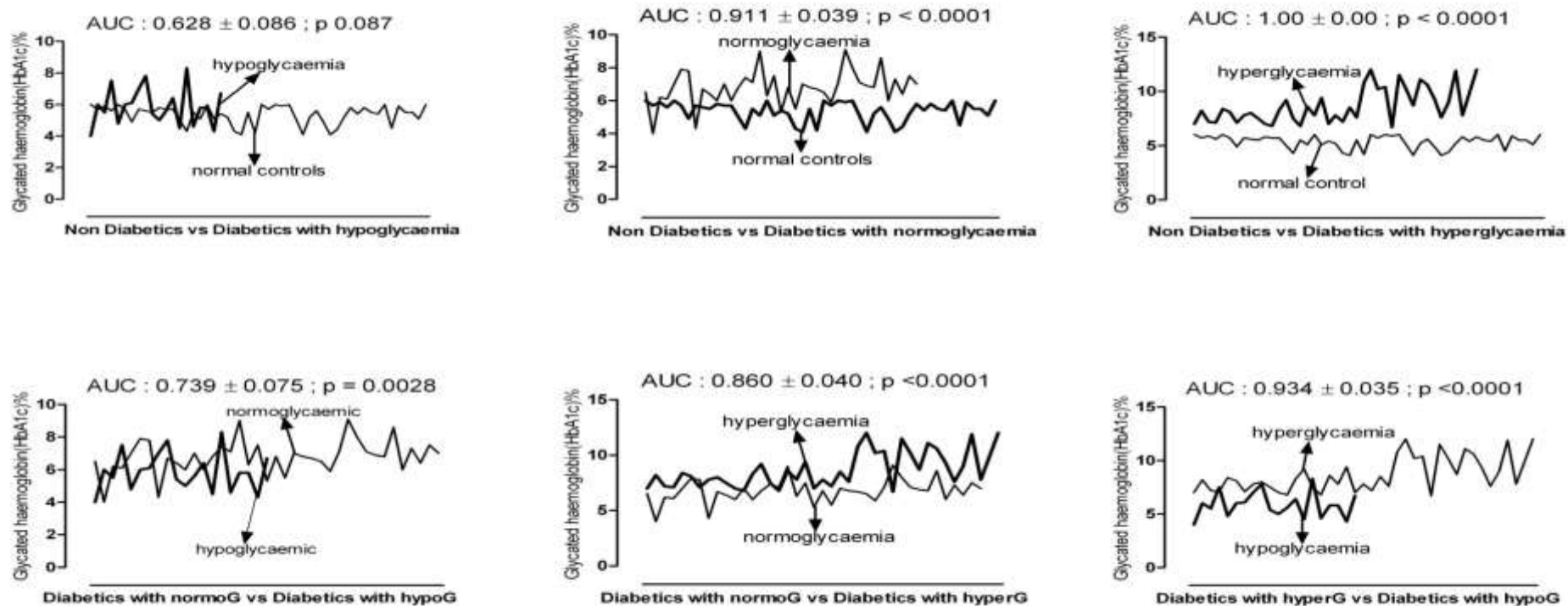


Figure 4-1: Relative Sensitivity And Specificity Of HbA1c Among Diabetics And Non Diabetic Subjects

4.10 LINEAR REGRESSION BETWEEN INDEPENDENT VARIABLE BLOOD GLUCOSE AND DEPENDENT VARIABLE GLYCATED HAEMOGLOBIN (FIGURE 4.3)

The graphs below represent linear regression analysis between independent variable blood glucose and dependent variable of glycated haemoglobin levels of the various glycaemic levels in diabetic and control population. At month one the average HbA1c of diabetics increased by $\beta = 0.4937 \pm 0.04409$, with coefficient of regression, R square (R^2) of 0.5613 ($p < 0.0001$) at a unit increase in blood glucose.

Graph of average HbA1c in month two increased by $\beta = 0.5154 \pm 0.04778$, $R^2=0.5428$, $P < 0.0001$ by a unit increase in blood glucose levels. Graph of HbA1c in month three increase by $\beta= 0.5455 \pm 0.04755$, $R^2 = 0.5731$, $P < 0.0001$ by a unit increase in blood glucose. The graph of linear regression between average HbA1c and mean fasting plasma glucose increased by $\beta = 0.5402 \pm 0.04596$, $R^2 = 0.5850$, $p < 0.0001$. Graph of HbA1c in control subjects increased by 0.168 ± 0.147 , $R^2 = 0.026$, $p > 0.05$ by a unit increase in blood glucose.

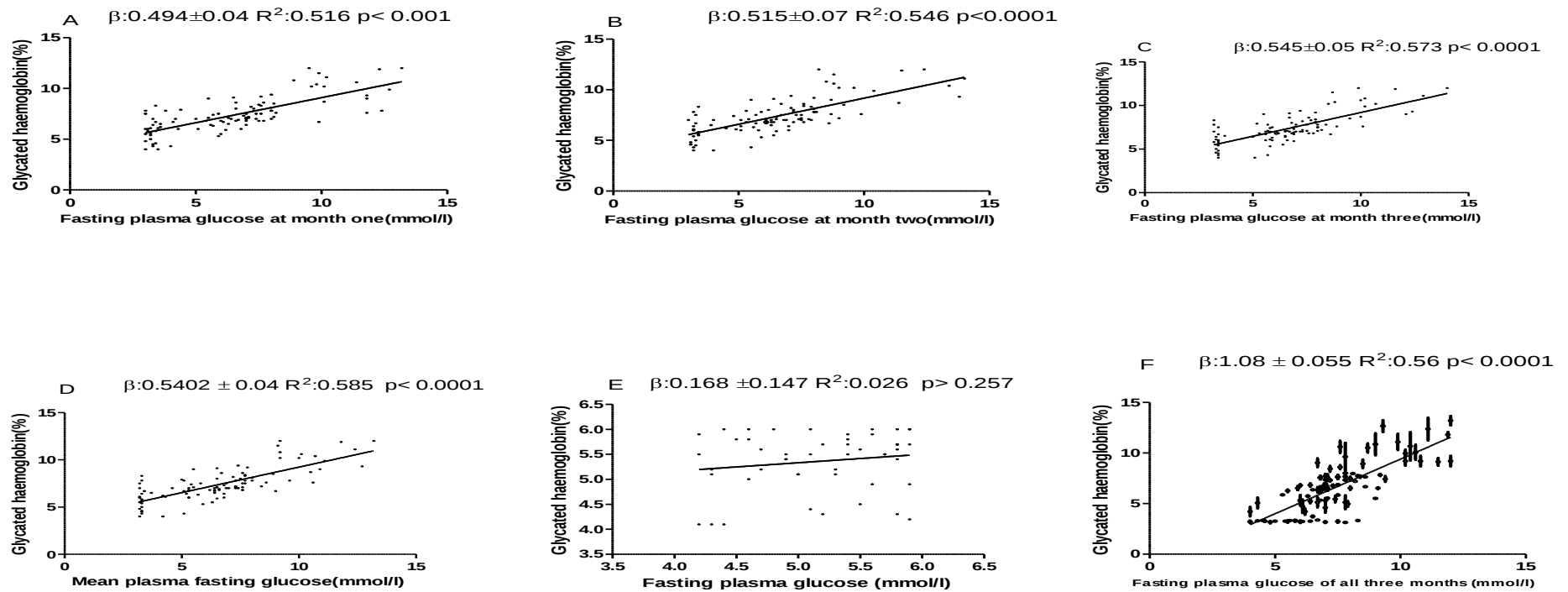


Figure 4-2: Linear Regression Graphs of Hba1c versus Monthly Fasting Plasma Glucose.

A. Average diabetic fasting plasma glucose at first month against average glycated haemoglobin. B. Average diabetic FPG at second month against average HbA1c. Average diabetic FPG at month three versus average HbA1c, D. Mean diabetic FPG versus HbA1c, E. Control mean FPG versus HbA1c, F. Fasting plasma glucose of all three months against HbA1c

Chapter 5

DISCUSSION, CONCLUSION & RECOMMENDATION

5.1 DISCUSSION

The only means of screening for diabetes or monitoring its treatment from this municipality is by determination of fasting plasma glucose concentration. Glycosylated haemoglobin measurement has been in existence for a long time. Fasting plasma glucose level estimation has its limitations like the person has to fast for a specified period of time (**Dandson and Schriger, 1999**). Similarly for random plasma glucose level the health care provider is not sure about the actual number of hours passed after the meal or any history of recent intake of any hypoglycemic or hyperglycemic drug (**Gillet, 2009**). HbA1c on the other hand, is a spot test with no requirement for prior preparation, risk of misinterpretation owing to recent meal or drug. The role and importance of glycated haemoglobin in the long-term assessment of diabetic patients has been recognized and appreciated (**Malik et al, 1996**). No data on blood glucose monitoring using glycated haemoglobin test as index for people with diabetes under treatment is available from the targeted area for the study.

High HbA1c concentration is associated with diabetic microvascular complications, macrovascular complications, risk of death, and cardiovascular disease. Positive correlations with metabolic syndrome are also suggested (**Malik et al, 1996**). HbA1c <6% is considered normal (**American Diabetes Association,**

ADA, 2008). The ADA-recommended treatment goal for the diabetic population is $\text{HbA1c} \leq 7\%$, although the target may be higher for older adults.

The study aimed at evaluating the use of glycosylated haemoglobin (HbA1c) test in monitoring treatment of diabetes mellitus, one of the commonest non-communicable diseases to affect man. It is the basis of treatment guidelines and is used universally to adjust therapy (**Dagogo-Jack and Santiago, 1997**). In order for patients to achieve HbA1c goals, they adjust their day-to-day therapies based on plasma glucose levels, measured with meters that are adjusted to provide values comparable to venous plasma levels (**Goldstein *et al*, 2004**). However, the relationship between HbA1c values and mean glucose levels has never been carefully explored, owing in great part to the absence of means to measure glucose levels frequently enough to reveal a complete description of mean glucose levels over time.

In this study the patients were recruited for measurement of the principal test (HbA1c) based on consistent FPG over the past three months as consistently hypoglycaemia, normoglycaemia and hyperglycaemia plasma glucose ranges from their medical records. The patients were all known diabetics who were undergoing treatment at Dormaa Presbyterian hospital in the Brong Ahafo region. We used monthly fasting plasma glucose monitoring in patients with diabetes with relatively stable glycaemia, over three month period and in non-diabetic

individuals, to determine true mean glycaemia. HbA1c assays were measured and best fit correlations determined overtime.

There were 65 females and 35 males recruited under the diabetic population. The female population was almost twice the number of the male counterpart. This compares well with a study on WHO global data (**WHO, 1998**) which stated that the prevalence ratio of diabetes between men and women varies markedly, with no consistent trend. However, impaired glucose tolerance is more common in women than in men. Association between the instantaneous fasting blood glucose was observed across gender. However it was generally inferred from the study that protein glycation occurs more higher among male subjects as strength of correlation was higher than their female counterparts. The relative difference in frequency between the sexes is probably related to the presence of underlying factors, such as pregnancy and obesity, rather than to a sex-specific genetic tendency (**American Diabetes Association, 2008**). From this study, it was observed that the diabetic subjects were averagely older. This shows type 2 diabetes begins typically in middle life or later, the prevalence rises with age. This is consistent with studies published by **WHO (1998)**. This also implies that impact of age as a risk factor of diabetes cannot be overemphasized as this trend has been demonstrated in most study populations around the world (**Ford *et al*, 2002**).

The American Diabetes Association (ADA) recommends body mass Index 19–24.9 is taken as normal, as defined by how much fat your body consists of as a

percentage of body weight while 25–30 is considered as overweight and BMI ≥ 30 is obesity (**Corpeleijn *et al*, 2008**). Weight gain is significantly associated with diabetes. The present study had diabetic patients and non diabetics having similar body mass indices, however mean values of diabetics (normoglycaemics and hyperglycaemics) were averagely higher than non diabetics except hypoglycaemics indicating that the patients were overweight; this overweight could be due to their different dietary habits.

From the study mean systolic blood pressure of diabetics and its systematic groupings were significantly higher as compared to the control group. However there was no significant difference between diastolic pressures of both diabetics and non - diabetics. Various studies have suggested the modulating effect of exercise on blood pressure (BP). BP tends to reduce in mean value as the physical activity increases. This has strong association with cardiac events and decreased fasting glucose (**Perry *et al*, 1994**).

Blood glucose is a continuous variable, rising and falling about two-fold throughout the day in people without diabetes, and up to some 10-folds in people with diabetes (**Saudek *et al*, 2005**). This study saw no significant difference in the fasting plasma glucose of the study diabetics when compared to the controls. This shows that diabetics on treatment can be as healthy as people without the condition. However their HBA1c levels can serve as the discriminant variable

The mean haemoglobin values of all diabetic groups showed highly significant decreases as compared to non - diabetics. The present study therefore extends these findings to diabetic patients with modest renal impairment. In diabetes, anaemia, associated with erythropoietin deficiency, appears to occur earlier in the course of progression of kidney disease than in non-diabetic patients with kidney diseases (**Valle *et al*, 1999**). Autonomic neuropathy has been postulated to play a role in erythropoietin dysregulation in diabetic patients (**Spallone *et al*, 2004**). In addition, nutrient deficiencies including iron, folate, and vitamin B12, or undetected malignant diseases or anaemia of chronic inflammation (formally termed anaemia of chronic diseases) may contribute to anaemia in diabetic subjects (**Guralnik *et al* , 2004**). The mechanisms by which lower haemoglobin levels may impact on the progression of chronic kidney disease in type 2 diabetes are unknown. Some of the explanations may be provided by in vitro and in vivo studies examining the effects of anaemia-induced renal hypoxia and oxidative stress.

Before this study none of the clients had tested for glycosylated haemoglobin (HbA1c). The complications of both Type 1 and 2 Diabetes do not develop or progress when the average HbA1c level is kept at < 7% (**Brownlee and Hirsch, 2006**). The present study found mean levels of HbA1c among diabetics were significantly higher as compared to the control group. A normal HbA1c averages 6 percent or less and an average of 6.5 percent or more indicates diabetes. Hyperglycaemia, or high blood sugar, increases the percentage of glycosylated

haemoglobin. The reverse is also true; hypoglycaemic episodes will reduce HbA1c readings but may not reflect proper management of diabetes. Normal or low HbA1c levels in a diabetic patient may indicate the use of too much insulin (**Mokdad *et al*, 2000**) as present in this study.

The direct relationship of age with fasting plasma glucose, and glycated haemoglobin were observed in both sexes of diabetic group. This shows that as age increases glycation also rises in diabetics. This is consistent with studies done by **Tahir *et al*, (2008)**, which found positive linear relation with age of diabetics and HbA1c. There is therefore a significant correlation between HbA1c and age of the patients.

The present study on applying pearson product correlation found a highly significant correlation between monthly fasting plasma glucose, mean fasting glucose and glycated haemoglobin (HbA1c) levels $p < 0.0001$ as shown in Table-4.3 in both male and female diabetics. The strongest positive correlation between HbA1c and blood glucose level was for the mean FPG (MFPG) for the three month period. Biosynthesis of glycosylated haemoglobins (HbA1c) occurs slowly, continuously and almost irreversibly throughout the three month life span of erythrocytes and the process is wholly non-enzymatic. The strongest correlations between HbA1c and Mean plasma glucose (MPFG) levels appeared to be over the preceding 12 weeks, consistent with prior studies (**Gillet, 2009**). Of note, although there was less than a perfect correlation between the measured MPFG and the

HbA1c levels, the relatively high correlation coefficient values, especially given the limited sample size and range of values, suggests that there are probably no extraneous factors that affect the relationship between MPFG and HbA1c in a substantive way. Not all cell types in the body require insulin for the uptake of glucose. Red blood cells have 'insulin-independent' glucose transporters on their surface. So if glucose levels in the plasma (the watery part of the blood) are high, then glucose levels inside the red blood cells will also be high. The higher the blood glucose level, therefore, the more glycosylation of haemoglobin will occur (**Chalew et al, 2010**). Some studies have suggested that variable red cell turnover, which may be affected by hyperglycemia, may alter the relationship between MPFG and HbA1c values (**Virtue et al, 2004**,). We found in Type 2 diabetes mellitus a good correlation between HbA1c and MPFG, as previously described in other studies (**Koga et al, 2006**). **Nielsen et al (2007)** showed that mean and standard deviation of glucose hyperglycaemic and hypoglycaemic states, all correlate with HbA1c, but with decreasing statistical power. This is consistent with the present study. In linear regression analysis, graph of HbA1c were plotted against monthly fasting plasma glucose as well as mean fasting glucose for the whole three months period. The corresponding coefficient of regression (goodness of fit)(R^2) were as follows 0.5613, 0.5428, 0.5731, 0.5850, for first, second, third months and mean fasting plasma glucose respectively. It was also found that mean fasting glucose gave the highest coefficient of regression than the monthly fasting plasma glucose. In this study it is clear that a close relationship exists between HbA1c and mean blood

glucose. The rate of formation of HbA1c is directly proportional to the ambient blood glucose concentration. This is consistent with the observation of **Watkins, (1993)**. This positive correlation shows that HbA1c is a useful index in the retrospective assessment of glucose control in diabetes. This is consistent with **Alexandria (1996)** studies which stated that HbA1c be interpreted as an average of the blood glucose present over the past 3-4 months. Thus glycosylated haemoglobin provides an accurate and objective measure of glycaemic control over a period of weeks to months as reported by **Malik *et al.*, (1996)**.

This means glycated haemoglobin is dependent on blood glucose concentration. As HbA1c increases there is a unit increase in blood glucose levels especially in the normoglycaemic and hyperglycaemic range but not in hypoglycaemic range. Thus both types of patients, i.e. those exhibiting controlled glycaemic (normoglycaemic) as well as patients with seemingly stable elevated glycaemic levels (hyperglycaemia), displayed poor long term regulation in terms of HbA1c suggesting that both duration and amplitude of hyperglycaemia have an impact on glycosylation. Thus there were significant regression from the corresponding HbA1c ($p < 0.05$). There exist a good agreement between monthly fasting plasma glucose, mean fasting glucose and glycosylated haemoglobin values for diabetics when applying linear regression analysis but insignificant between the controls blood glucose as shown in figure 4.2. This is consistent with observation derived from **Akinloye *et al* (2007)**. In a study conducted in China, **Hu *et al* (2009)** recommended combined use of random plasma glucose, RPG and FPG for the

monitoring of diabetes while an expert committee comprising members appointed from the American Diabetes Association (ADA), the European Association for the Study of Diabetes (EASD) and the International Diabetes Federation (IDF) in 2008 have discouraged the combined use of these two tests and has proposed adoption of only HbA_{1c} for monitoring diabetes.

The sensitivity of glycated haemoglobin to detect threshold between diabetics and non diabetics was further investigated by plotting a relative operative characteristics graph curve (ROC) as seen in figure 4.1. It was found that HbA_{1c} is more sensitive towards hyperglycaemia. This means high blood glucose in diabetics will mean high concentration of HbA_{1c} and consequent micro and macrovascular complications as previously described by **Lauritzen, (1985)**. In conditions of sustained hyperglycemia, such as in diabetes mellitus, the proportion of haemoglobin that is glycated is increased substantially (**Rahbar, 1968, Trivelli, et al, (1971)**). For patients and health care providers, a clear understanding of the relationship between plasma glucose (PG) and HbA_{1c} is necessary for setting appropriate day-to-day PG testing goals with the expectation of achieving specific HbA_{1c} targets. Several studies have suggested that, although intraindividual variation in HbA_{1c} is minimal, there is evidence of wide fluctuations in HbA_{1c} between individuals that are unrelated to glycaemic status, suggesting that there are "low glycaters" and "high glycaters". **Riet et al, (2010)** demonstrated strong correlations between glucose and HbA_{1c} in known diabetics; however, moderate correlations were found in the general population. This is consistent with this

study where all the subjects recruited were under treatment for this chronic disease but the patients fasting blood glucose correlated significantly with HbA_{1c}. The resulting strong correlation suggests that, although a single blood glucose (BG) measurement may not reliably predict HbA_{1c}, BG, levels measured over time can provide a reasonably accurate estimation of HbA_{1c}.

In diabetes, anaemia, associated with erythropoietin deficiency, appears to occur earlier in the course of progression of kidney disease than in non-diabetic patients with kidney diseases (**Thomas *et al*, 2004**). Haemoglobin concentrations were found to be significantly decreased in patients with diabetes as compared to controls

The correlation between HbA_{1c} and haemoglobin in both male and female diabetics gave a negative non significant relationship (Table 4.4). The relevance of haemoglobin levels was not determined in this study when correlated with glycated haemoglobin. Other studies by **Rossing *et al*, (2004)** have demonstrated that decreased haemoglobin levels may be useful in identifying type 2 diabetic individuals at increased risk of progression of kidney disease and also anaemia complications of diabetes mellitus.

However studies done by **Azim *et al*, (2010)** found no correlation between age of the patients and the HbA_{1c} or fasting plasma glucose. Studies conducted by **Arnetz *et al* (1984)** and **Kilpatrick *et al* (1996)** in diabetic patients have shown a significant positive correlation between HbA_{1c} and age as well as duration of

diabetes. In contradiction to this **Kabadi (1998)** found no significant relationship between age, duration of diabetes and fasting blood glucose (FBG), and glycated haemoglobin. In this study we found negative significant correlation between the duration of disease and HbA1c probably as duration of treatment of disease increases the better the glycaemic control (Table 4.4).

This study found no significant correlation between HbA1c and BMI as shown in Table 4.4. The ADA recommends patients with diabetes screen for microalbuminuria which has significant correlation with HbA1c as independent risk factor for diabetic nephropathy (**BrownLee & Hirsh, 2006**).

Diabetes mellitus and hypertension have strong associations, suggesting an increase in severity of coronary artery disease in the presence of either of the factors. Higher levels of haemoglobin A1c (HbA1c) and blood pressure precede the development of nephropathy in Type 1 diabetes (T1DM) (**Diabetes Control and Complications Trial Research Group, DCCT, 1993**). Weak positive correlation existed between HbA1c and systolic and diastolic blood pressures from the table 4.2 and table 4.4. Previously, the Diabetes Control and Complications Trial (**DCCT, 1993**) had shown that improved glycaemic control in type 1 DM is associated with a sustained decrease in microvascular, macrovascular and neuropathic complications. Previous studies like, **Chalew et al, (2010)** found no correlation between mean HbA1c and systolic blood pressures. It however found significant correlation between diastolic pressure and HbA1c.

Correlation between HbA1c and anthropometric, clinical and laboratory parameters of non-diabetics reveal interesting relations (Table 4.5). From the table, HbA1c showed a significant negative correlation with age but gave positive non significant relation with fasting plasma glucose. HbA1c however gave negative non significant correlation with body mass index, systolic, diastolic blood pressures and haemoglobin levels. This shows that HbA1c measurement is useful in known diabetics.

Correlation between mean fasting plasma glucose versus anthropometric, clinical and laboratory indices of diabetic subjects reveal direct relationship exist between FPG and age, as well as systolic blood pressures. On simple correlation fasting plasma glucose was a positive function of age. This is also in agreement in previous studies. **Liesenfield *et al*, (1996)** found that fasting serum glucose increases as age increases. In the present study, BMI showed a positive correlation with FBS. A positive correlation between BMI and blood sugar was also reported by other studies (**Adamu *et al*, 2006**). The observed positive correlation between BMI and FBS reiterates the diabetogenic effect of adipose tissue and emphasizes the importance of the maintenance of normal BMI to prevent the early onset of diabetes complications. This correlation was similarly observed by **Vittal *et al*, (2010)** on relationship between BMI and fasting blood glucose among healthy individuals. It however gave negative significant relationships with haemoglobin and duration of disease (Table 4.6)

Mean fasting plasma glucose however gave inverse relation with age, systolic, diastolic and haemoglobin parameters of non diabetics which was not significant. It however gave positive non significant correlation with BMI for non - diabetics.

5.2 CONCLUSION

Stable FPG means predictable mean glycaemia which also implies predictable HbA1c. Clearly long term glycaemic control should be based on mean glycaemia and not on an instant plasma glucose concentration. Also mean glycaemia has implications for the prognosis of diabetes mellitus. The poorer the glycaemic control, the poorer the prognosis. This is because protein glycation which underlies a lot of the diabetic complications proceeds at a faster rate under poor glycaemic control. Mean glycaemia strongly correlates with HbA1c be reliably depended on as an index of prognosis of diabetes mellitus. During treatment, for good prognosis the goal must be to keep HbA1c less than 7%. In this study it can be deduced that HbA1c can be used to determine average glycaemic control, determination of prognosis and for quality assurance in management of this chronic disease at the clinic. Findings of other anthropometric, clinical and laboratory parameters also suggest that improved glucose control could result from tight control and life style changes in the management of the disease.

5.3 RECOMMENDATION

Further studies with a larger sample size and probably long follow- up are necessary to validate sensitivity of HbA1c levels as well as other parameters measured in this study to clarify the etiology of various findings for better management of diabetic cases.

HBA1c testing should be incorporated into the management of diabetic patients in Dormaa Ahenkro Presbyterian Hospital.

REFERENCES

Adamu GB, Geoffrey CO, Bala GS, Ibrahim SA, Sani SH, Tambaya MA (2006). Relationship between random blood sugar and body mass index in an African population. *Int J Diabetes & Metabolism*; 14: 144-5.

Akinloye OA, Akinlade KS, Odetola AA, (2007). Relationship between Fasting Plasma Glucose and Glycated Haemoglobin In Adult Diabetic Nigerians. *African Journal of Biomedical Research*; 10:127-132.

Alberti K. G. and Zimmet, P. Z., (1998) Diabetes Non-enzymatic glycosylation of lysine residues post translation. *Diabetologia* Volume 25,496 -501. *Med.15*, 539–553.

Alexandria V.A. (1996), American Diabetes Association ‘Complete Guide to Diabetes, American Diabetes Association, pp 124 – 138.

American Diabetes Association (2003), Diagnosis and classification of diabetes mellitus. *Diabetes Care*.;31 Suppl 1:S55-60.

American Diabetes Association (2008) Diagnosis and classification of diabetes mellitus. *Diabetes Care*. Jan ;31 Suppl 1:S55-60.

Amoah A. G., Owusu S. K., Adjei S. (2002), Diabetes in Ghana: A Community Based Prevalence Study in Greater Accra. *Diabetes Research and Clinical Practice*. 56:197–205.

Arnetz, B.B, Kallner, A. and Theorell, T (1982). The influence of aging on HbA1c. *Journal Gerontol*; 37: 648-650.

ATAC 8000 Random Access Chemistry analyzer, Elan Diagnostics, Thurber Boulevard, Smithfield R102917, USA.

ATAC operators manual (2007). Elan ATAC 8000 Random access Chemistry analyser.

Aubert RE, Herman WH, King H, (1998): Global burden of diabetes from 1995 – 2025: Prevalence, numerical estimates and projections. *Diabetes care*; 21:1414 – 1431.

Azim W, Omair M, Khan MQA, Shaheen N, Azim S , (2010) Correlation between Glycated Haemoglobin and Random Plasma Glucose Levels for the Screening of Diabetes Mellitus *International Journal of Pathology*; 2010; 8(2): 59-62.

Baynes J.W., Thorpe SR, Mustiashow Mt. (1984), Non-enzymatic glycosylation of lysine residues post translation. *Diabetologia* Volume 25, 496 - 501.

Bergenstal RM, Gavin JR, 3rd. (2005) The role of self-monitoring of blood glucose in the care of people with diabetes: report of a global consensus conference. *American Journal Medicine* 118 (Suppl 9A): 1S-6S.

Bookchin RM, Gallop PM (1968). "Structure of haemoglobin A1c: nature of the N-terminal beta chain blocking group". *Biochem. Biophys. Res. Commun.* 32 (1): 86-93.

Boule NG, Kenny GP, Haddad E, Wells GA, Sigal RJ. (2003) Meta-analysis of the effect of structured exercise training on cardiorespiratory fitness in Type 2 diabetes mellitus. *Diabetologia* 46(8): 1071-81.

British Medical Journal (1980), Haemoglobin A1 and Diabetes mellitus, - a reappraisal. Editorial, *Diabetologia*, pp 1304-5

Brown Lee M, 1990, Advanced products of nonenzymatic glycosylation and pathogenesis of diabetic complication .*New England Journal. Med.*318, 1315-21.

Brownlee M, Hirsch IB. (2006) Glycaemic variability: A haemoglobin A1c-independent risk factor for diabetic complications. *JAMA*;295 (14):1707-8.

Bry L, Chen PC, Sacks DB. (2001) Effects of haemoglobin variants and chemically modified derivatives on assays for glycohaemoglobin. *Clinical Chemistry* 47 (2): 153-63.

Bunn, F, Haney, D. N., Gabby, K. H. and Gallop, P. M., (1975). Biochemistry, Biophysics. *Res. Commun*, **67**, 103.

Cahill GF, Etzwiler DD, Freinkel W (1976): Control and diabetes.*N Engl J Med*, 294, 10045.

Ceriello A, Giugliano D, Quatraro A, Donzella C, Dipalo G, Lefebvre PJ. (1991) Vitamin E reduction of protein glycosylation in diabetes. New prospect for prevention of diabetic complications? *Diabetes Care* 14(1): 68-72.

Chalew SA, McCarter RJ, Hempe JM, (2010), Mean blood glucose and biological variations have greater influence on HbA1c levels than glucose

instability: An analysis of data from the Diabetes Control. Metab Analysis 29-32.

Chandalia, H. B., Nomani, H. and Krishnaswamy, P. R, (1988) in Diabetes Research and Clinical Practice Elsevier, Amsterdam, Abstract No. POS-001-149, supplement 1-S-152-

Chatured N, John J, Nick Morrish (1996) ;Differences in mortality and morbidity in African Caribbean and European people with NIDDM, British Medical Journal volume 313.

Cheesbrough M, (2002), District laboratory practice for Tropical countries, Part 1 Cambridge University press 330 -340

Chen ET, Nichols JH, Duh SH, Hortin G. (2003) Performance evaluation of blood glucose monitoring devices. Diabetes Technol Ther 5(5): 749-68.

Corpeleijn E, Mensink M, Kooi ME, (2008). Impaired skeletal muscle substrate oxidation in glucose-intolerant men improves after weight loss. Obesity (Silver Spring) 16(5):1025-32.

Dagogo-Jack S, Santiago JV (1997). Pathophysiology of type 2 diabetes and modes of action of therapeutic interventions. Arch Intern Med. ;157(16):1802-17

Dandson MB, Schrager D.L (1999) Relationship between fasting plasma glucose and glycosylated haemoglobin. Journal of the American Medical Association 1999,281 (13): 1203 -10.

Davie SJ, Gould BJ, Yudkin JS. (1992) Effect of vitamin C on glycosylation of proteins. *Diabetes* 41(2): 167-73.

DCA 2000+ HbA1c analyzer, Elkart, IN 46515 Bayer Corporation, USA.

Diabetes Control and Complications Trial Research Group (1993), The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus, *N Engl J Med*. Sep 30 ;329(14):977-86.

Diabetes Management by Aventis, (2006).

Falko JM, O'Dorisio TM, Cataland S. (1982) Spurious elevations in glycosylated haemoglobin (HbA1) secondary to hypertriglyceridemia. *Arch Intern Med* 142(7): 13701.

Fluckiger, R. and Winter halter, K. H., (1976) Pathogenesis of pre-diabetes: mechanisms of fasting and postprandial hyperglycemia in people with impaired fasting glucose and/or impaired glucose tolerance. *Diabetes*. 3536-49
FEBS Letts, 71, 356.

Ford, E.S Giles, W.H. and Diez, W.H. (2002) Prevalence of metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA* 287:356-359.

Frier BM, Fisher BM, (1999) eds. Hypoglycaemia in clinical diabetes. Chichester: John Wiley pp 342-46.

Garber AJ, Wahlen J, Wahl T, Bressler P, Braceras R, Allen E, Jain R. (2010)

Attainment of glycaemic goals in type 2 diabetes with once-, twice-, or thrice-daily dosing with biphasic insulin as part 70/30 (The 1-2-3 study). *Diabetes Obesity Metabolism* 8(1): 58-66.

Garrib A, Griffiths W, Eldridge P, Hatton R, Worsley A, Crook M. (2003)

Artificially low glycated haemoglobin in a patient with severe hypertriglyceridaemia. *Journal Clinical Pathology* 56(5): 394-5.

Gill, G. V. (1997), "Outcome of Diabetes in Africa." In *Diabetes in Africa*, ed. G. V. Gill, J.-C. Mbanya, and K. G. M. M. Alberti, 65-71. Cambridge: FSG Communications.

Gillet MJ (2009). Guidelines Review-International Expert Committee Report on the Role of the A1C Assay in the Diagnosis of Diabetes *Diabetes Care*; 32(7): 1327-1334.

Goldstein DE, Little RR, Lorenz RA, Malone JL, Nathan D, Peterson CM, Sacks DB. (2004) Tests of glycaemia in diabetes. *Diabetes Care* 27(7): 1761-73.

Goldstein DE, Little RR, Wiedmeyer HM (1986) Glycated Haemoglobin: Methodologies and Clinical applications *Clinical Chemistry*.32, B64 – B.

GraphPad software, San Diego California USA, www.graphpad.com

Green A, King H, Rogle G, Sicree R, and Wild S., (2004), Global prevalence of Diabetes, estimates for the year 2000 and projections for 2030, *Diabetes Care*, 27 1047-1053.

Guralnik JM, Eisenstaedt RS, Ferrucci L, Klein HG, Woodman RC (2004) Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. *Blood* 104:2263–2268.

Guthrie, Diana W (2010), *The Diabetes Sourcebook: Today's Methods and Ways to Give Yourself the Best Care*, 3rd edition. Los Angeles: Lowell House.

Henry RT, Cannon DC, Winkerman JW, (1974), *Clinical chemistry, principle and Techniques* 2 nd edition 504-06.

Hoffman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. (2008) 10-year follow-up of intensive glucose control in type 2 diabetes. *New England Journal Medicine* ,359(15): 1577-89.

Holmquist, W. R. and Schroeder, W. A (1966), *Harrison's Principles of Internal Medicine*. 17th ed. New York, NY: McGraw-Hill; 5,2489.

Hu Y, Liu W, Chen Y, Zhang M, Wang L, Zhou H, Wu P (2009). Combined use of fasting plasma glucose and glycated haemoglobin A1c in the screening of diabetes and impaired glucose tolerance. *Acta Diabetologica*.

Huisman TH, Martis EA, Dozy A (1958). "Chromatography of haemoglobin types on carboxymethylcellulose". *Journal Laboratory Clinical Med.*52 (2): 312–27.

Hunter J. M., Sparks B. T., Mufunda J., Musabayane C. T., Sparks H. V., Mahomed K. (2000) *Economic Development and Women's Blood Pressure:*

Field Evidence from Rural Mashonaland, Zimbabwe. Social Science and Medicine. 50:773-95.

International Diabetes Federation (2003) Access to Insulin: A Report on the IDF Insulin Task Force on Insulin, 2001 - 2002 Brussels.

Iyer, P. D. and Krishnaswamy, P. R. (1980), Impaired fasting glycaemia vs impaired glucose tolerance: similar impairment of pancreatic alpha and beta cell function but differential roles of incretin hormones and insulin action (5):853-861, Jaslok Hospital Bullet., 5, 17-19.

Kabadi, U.M (1998). Glycosylation of proteins. Lack of influence on ageing. Diabetes Care;11: 421-432.

Kilpatrick, E. S. Peterson, K. P., Pavlovich, J. G., Goldstein, D (2000), Tests of glycaemia in diabetes. Diabetes Care, 27, 91-93. British Medical Journal 309, 983-986.

Kilpatrick, E.S., Dominiczak, M.H. and Small, M (1996). The effects of ageing on glycation and the interpretation of glycemic control in type 2 diabetes. Q.J. Med; 89: 307-312.

King, H. and Rewers M. (1993) Worldwide experience of metformin as an effective glucose lowering agent: a meta-analysis, Diabetes Care, 16, 157-177.

Koenig RJ, Peterson CM, Jones RL, Saudek C, Lehrman M, Cerami A (1976)."Correlation of glucose regulation and haemoglobin A1c in diabetes mellitus". New England Journal Med.295 (8): 417-20.

Koenig RJ, Peterson CM, Jones RL, Saudek C, Lehrman M, Cerami A (1976).

"Correlation of glucose regulation and haemoglobin A1c in diabetes mellitus".
New England Journal (8): 417-20.

Koga, M., Matsumoto, S., Saito, H., Kasayama, S (2006). A guide for the diabetics in the elderly, *Endocrine J*, 53, 387-391.

Kruseman AC, Mercelina L, Degenaar CP. (1992), Value of fasting blood glucose and serum fructosamine as a measure of diabetic control in non-insulin-dependent diabetes mellitus. *Horm Metab Res Suppl* 26: 59-62.

Lauritzen T, Forst-Larsen K, Larsen HV, Deckert T (1985). Steno Study group: Two-year experience with continuous subcutaneous infusion in relation to retinopathy and nephropathy. *Diabetes*; 34 (suppl. 3): 74-9.

Liesenfield Peters AL, Davidson MB, Schringer DL, Hasselblad V. A (1996)
Clinical Approach for the Diagnosis of Diabetes Mellitus. An Analysis Using Glycosylated Hemoglobin Levels.; Division of Clinical Biochemistry, Institute of Medical and Veterinary Research UK

Liu, Q. Z. Franks PW, Hanson RL, Knowler WC, (1993), Glucagon-like peptide 1 (GLP-1) affects basic caused of the disease, *Diabetologia*, 36, 428-432.

Lyons, T. J., Silvestri, G., Dunn, J. A. and Baynes, J. W., (1991) Glucose intolerance and cardiometabolic risk in adolescent exposed to maternal gestational diabetes - a 15-year follow-up study. *Diabetes Care*. *Diabetes*, 40, 1010-1015.

Malik, M., Gill, G.V., Heynigden, C.V., Pugh, R.N. (1996). Assay Methods for glycated haemoglobin: A review for tropical hospitals International Diabetes Digest, 7: No.1 Jan.

McIntosh, A., Hutchinson, A., Home, P. D., (2001) Clinical Guidelines and Evidence Review for Type 2 Diabetes: Management of Blood Glucose. Sheffield, UK. Scharr, University of Sheffield British Medical Journal.

Medical University of Ohio (2009), The effects of biofeedback-assisted relaxation on HbA1c in those with type 2 diabetes, Diabetes care, 912-914.

Microtech 3000 spectrophotometer, Vital Scientific N.V.P O Box 100,NL 6950 AC Dieren ,The Netherlands.

Modan, M., Meytes, D., Rozeman, P., Yosef, S. B., Sehayek, E.and Yosef, N. B. (1988), Mortality by Cause for Eight Regions of the World: Global Burden of Disease Study. Lancet. 349:1269–76. *ibid*, 11, 422–428.

Mokdad AH, Ford ES, Bowman BA, Dietz WH, Vinicor F, Bales VS, (2001). Prevalence of obesity, diabetes, and obesity related health risk factors. 289:76–9.

Mokdad AH, Ford ES, Bowman BA, Nelson DE, Engelgau MM, Vinicor F, Marks JS. (2000) Diabetes trends in the U.S.: 1990-1998. Diabetes Care 23(9): 1278-83.

Monnier L, Lapinski H, Colette C. (2003) Contributions of fasting and postprandial plasma glucose increments to the overall diurnal hyperglycaemia

of type 2 diabetic patients: variations with increasing levels of HbA1c. Diabetes Care 26 (3): 881-5.

Monnier, V. M., Vishwanath, V., Frank, K. E., Elmets, C. A., Douchat, P. and Kohn, R. R(1986), The glycosylation of haemoglobin: relevance to Diabetes Mellitus. Science 200(4337): 21-7. New England Journal., 314, 403-411.

Murray C. J. L., Lopez A. D. (1997) Mortality by Cause for Eight Regions of the World: Global Burden of Disease Study. Lancet. 349:1269-76.

National Committee for Quality Assurance (1999): Technical Specifications. Washington DC: National Commission for Quality Assurance pp 105-09.

Neilsen JK. Sharp P, Rainbow S.(2007); Continuous Subcutaneous Glucose Monitoring Shows a Close Correlation between Mean Glucose and Time Spent in Hyperglycemia and Hemoglobin A1c. J Diabetes Sci Technol. 1(6):857-863.
17- Sharp P, Rainbow S. Continuous glucose monitoring

Owusu SK., (1976) Diabetes in Ghana – A 10 year study; Ghana Medical Journal: 15; 93 -96.

Panzer S, Kronik G, Lechner K, Bettelheim P, Neumann E, Dudczak R. (1982) Glycosylated haemoglobins (GHb): an index of red cell survival. Blood 59(6): 1348-50

Perry, I.J., Whincup, P.H. and Shaper, A.G. (1994). Environmental factors in the development of essential hypertension. British Medical Bullets 50:246-259.

Population and Housing census (2000). Ghana Statistical Service, population density and indicators by region, overview pp 45-48.

Rahbar, S (1968). An abnormal haemoglobin in red cells of diabetics. Clin. Chim. Acta; 22:296- 298.

Ravel R, (1984), Test of Diabetes and Hypoglycaemia, Clinical laboratory medicine pp 235 -239

Reaven GM, (1988); Role of insulin resistance in human disease, Diabetes 37: 1595-1607

Riet EW, Alsema M, Rijkelijhuizen JM, Kostense PJ, Nijpels G, Dekker JM (2010). Relationship between A1c and Glucose Levels in the General Dutch Population. Diabetes care; 33(1): 61-6. Body mass index calculator.

Rossing K, Christensen PK, Hovind P, Tarnow L, Rossing P, Parving HH (2004), Progression of nephropathy in type 2 diabetic patients. Kidney Int 66:1596–1605

Sacks DB, Bruns DE, Goldstein DE, Maclaren NK, McDonald JM, Parrott M (2002), Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clin Chem.; 48:436-472.

Saudek CD, Kalyani RR, Derr RL. (2005) Assessment of Glycaemia in Diabetes Mellitus: Haemoglobin A1c. Journal Association of Physicians India.; 53:299–304.

Shahbazkia, H.R., Nazifi, S., (2008) Complications of Diabetes, Essential Endocrinology, Comp Clin Pathol, 17, 9-12.

Spallone V, Maiello MR, Kurukulasuriya N (2004) Does autonomic neuropathy play a role in erythropoietin regulation in non-proteinuric Type 2 diabetic patients? *Diabet Med* 21:1174–1180.

Stanley B S, Frederick L, Donald Young S, (1982), Carbohydrate metabolism, Biochemical press.

Striker, G. E. Panzer S, Kronik G, Lechner K, Bettelheim P, (1993) Glycosylated haemoglobins (GHb): an index of red cell survival. *Blood* 59(6): 1348-50 *Diabetes Metabolism Review.*, 9, 37–56.

Stryer L, (1995,) Metabolism: Basic concept and Design, Biochemistry ed 5 W.H Freeman and Company New York pp 313 -331.

Tahir Saleem, Kazim Hameedullah Mohammad, Moataz M Abdel-Fattah and Abdul Hafeez Abbasi (2008), Association of glycosylated haemoglobin level and diabetes mellitus duration with the severity of coronary artery disease *Diabetes and Vascular Disease Research* 5: 184.

Tarim O, Kucukerdogan A, Gunay U, Eralp O, Ercan I. (1999) Effects of iron deficiency anaemia on haemoglobin A1c in type 1 diabetes mellitus. *Pediatric Int* 41(4): 357-62.

Thomas MC, MacIsaac RJ, Tsalamandris C (2004), Anaemia in patients with type 1 diabetes. *Journal Clinical Endocrinology Metabolism* 89:4359–4363.

Trivelli, L.A., Ranney, H.M. and Lai, H-T (1971). Haemoglobin components in patients with diabetes mellitus. *N. Engl. J. Med*; 284: 353-357.

Tuomilehto J., Lindstrom J., Eriksson J. G., Valle T. T., Hamalainen H., Ilanne-Parikka P., Keinanen-Kiukaanniemi S. (2001). Prevention of Type 2 Diabetes Mellitus by Changes in Lifestyle among Subjects with Impaired Glucose Tolerance. *New England Journal of Medicine*. 344:1343–50.

UKPDS (1998), Tight Blood Pressure Control and Risk of Macrovascular and Microvascular Complications in Type 2 Diabetes: 38 *British Medical Journal* 317:703–12.

Valenstein, P., Leiken A, Lehmann, C. (1988) Test-ordering by multiple physicians increases unnecessary laboratory examinations. *Archives of Pathology and Laboratory Medicine*, 112, 238–241.

Valle T, Koivisto V.A, Reunanen A, Kangas T, Rissanen A (1999) Glycaemic Control in Patients with diabetes in Finland. *Diabetes Care* **22** (4): 575-9.

Venkatraman, J., Shankaramma, S. C. and Balaram, P., Chem Rev., (2001), Microalbuminuria and Retinopathy in a Diabetic Population. *Diabetes Research and Clinical Practice*; 101, 3131–3152.

Virtue, M.A., Furne, J.K., Nuttall, F.Q., Levitt, M.D. (2004), Detection of an advanced glycosylation end product of bound to protein in situ, *Diabetes Care*, 27, 931-935.

Vittal B G, Praveen G, Deepak P, (2010) A Study Of Body Mass Index In Healthy Individuals And Its Relationship With Fasting Blood Sugar. *Journal of Clinical and Diagnostic Research*, volume 25:496-97.

Watkins, PJ. (1993): The ABC of Diabetes. 3rd edition. BMJ Publication group.

Weykamp CW, Penders TJ, Siebelder CW, Muskiet FA, van der Slik W. (1993) Interference of carbamylated and acetylated haemoglobins in assays of glycohemoglobin by HPLC, electrophoresis, affinity chromatography, and enzyme immunoassay. *Clinical Chemistry* 39(1): 138-42.

WHO (1997) Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*.20 (7):1183-97.

WHO (2003), The World Health Report, 2002 – Reducing Risks, Promoting Healthy Life. Geneva:

WHO (World Health Organization) 1998, Population Ageing—A Public Health Challenge. Geneva:

WHO Study Group (1985). Report of Diabetes mellitus. World Health Organ Tech Rep Ser. 1-113. *Diabetes Care*.30 (5):93-97.

Windeler J, Kobberling J. (1990) The fructosamine assay in diagnosis and control of diabetes mellitus pp 291-297.

Yudkin, J. S., Davis Louis MS, Jin K, (1996), Diabetic Nephropathy and Microalbuminuria in the community *Diabetes care*. 17, 1404-09.

