

*GLYCATED ALBUMIN AND GLYCATED HEMOGLOBIN  
LEVELS AS A MEASURE OF CONTROL IN DIABETIC  
PATIENTS ATTENDING OUT-PATIENT CLINIC AT  
KENYATTA NATIONAL HOSPITAL: a comparative study*

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A dissertation submitted to the University of Nairobi in part fulfillment of the requirements for the degree of Masters of Medicine in Human pathology.

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**Declaration**

I, Lotodo Teresa Cherop Loile, declare that this dissertation for Masters of Medicine in Human Pathology is my original work and has not to the best of my knowledge been presented by any other individual at any other institution of higher learning.

Signed.....

Date.....

**Supervisor Declaration**

This dissertation for the Master of Medicine in Human Pathology is submitted with our approval.

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## **Dedication**

This work is dedicated to my family, colleagues and friends.

## **Appreciation**

I humbly express my sincere gratitude to all those who either directly or indirectly contributed to the successful completion of this dissertation.

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## **List of Abbreviations**

**ADA**-American Diabetic Association

**ADAG**-A1C Derived Average Glucose

**A1C**-Glycated

**ACE**-American College of Endocrinology

**BMI**-Body mass index

**CAD**-Coronary Arterial Disorders

**DCCT**-Diabetes Control and Complications Trial

**DM**-Diabetes Mellitus

**DQA**-Beta chain of HLA complex

**DQB**-Alpha chain of HLA complex

**EDTA**- Ethylenediaminetetraacetic acid

**eGA**- Estimated Averaged Glucose

**EQA**- External Quality Assurance

**HbA1c**-Glycated hemoglobin

**HLA**-Human leukocyte antigen

**HPLC**-High performance liquid chromatography

**HUQAS**-Human Quality Assessment Service

**IGT**-Impaired Glucose Tolerance

**GA**-Glycated Albumin

**KNH**-Kenya National Hospital

**MCH**-Mean Corpuscular Hemoglobin

**MCV**-Mean Corpuscular Volume

**MEM**- Measurement Errors Mode

**MODY**- Maturity Onset Diabetes of the Young

**NGSP**-National Glycohemoglobin programme



**OGLA**-Oral glucose lowering agents

**RBS**-Random Blood Sugar

**RBC**-Red Blood Cells

**ROC**-Receiver operating characteristic curve

**SMBG**-Self Monitoring of Blood Glucose

**SPSS**-Statistical packages for the social sciences

**UKPDS**-United Kingdom Prospective Diabetes Study

**Va/DoD**- Veteran affairs department of defence

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## **Summary**

### **Background**

Diabetes Mellitus is a chronic metabolic disease that is characterized by persistent hyperglycemia. Progression of uncontrolled Diabetes Mellitus (DM) causes complications in several organs. Glycaemic control in individuals with DM is currently done by a combination of short term and long term biochemical tests. HbA<sub>1c</sub> represents time averaged plasma glucose level over 2-4 months while Glycated Albumin is a time averaged plasma glucose level over two to four weeks. The latter enables closer monitoring and evaluation of treatment regimen faster.

### **Broad Objective**

To compare glycated albumin to glycated hemoglobin levels as a measure of glycemic control for Diabetic patients attending outpatient clinic at Kenyatta National Hospital.

### **Specific Objectives**

1. To determine random blood glucose in both type 1 and type 2 diabetic patients.
2. To determine levels of glycated albumin and glycated hemoglobin (HbA<sub>1c</sub>) in the study population.
3. To correlate the glycated albumin and glycated hemoglobin levels in the study population.

### **Methodology**

**Study design:** Comparative cross-sectional descriptive study

**Study area:** Diabetic clinic at Kenyatta National Hospital

**Study population:** Diabetic patients both type 1 and 2 attending the diabetic clinic

**Sampling procedure:** Diabetic patients were assessed for eligibility and recruited into the study. Recruitment was done consecutively till the desired number was achieved. Files for the eligible

patients were perused through to check for documented complications. A questionnaire was filled for the eligible patients and 4 ml of venous blood taken for analysis.

### **Laboratory Analysis:**

After recruiting the study subjects, blood samples were collected (4 ml) from the consenting patients. Test for random blood sugar was done using a glucometer, Glycated Albumin and HbA1c were also determined and data collected and analyzed. Other characteristics like type of diabetes, body mass index and type of treatment the patients were using were also noted. The performance of the two tests was compared against each other.

### **Data Management:**

The data obtained from the laboratory was entered into a computer database. Spreadsheets were generated and analyzed using windows *SPSS version 17*

### **Results**

The mean age was 52 yrs with a female preponderance of 60.4%. Majority of the patients had type 2 diabetes, 214 (82.3%), while only 46 (17.7%) had type 1. A large number of patients were on insulin, 100 (38.5%) followed by those on oral glucose lowering agents, mainly metformin. Random blood sugar analysis showed that, the population with good glycemic control constituted 156 (60%). Results from this study showed that majority of the patients had good glycemic control 170 (65.4%) based on the HbA1c assay, compared to 39.4% in GA. In this study there was no correlation between Random blood sugar with either HbA1c or Glycated Albumin. There was correlation between HbA1c and Glycated Albumin with  $R^2$  value of 0.64.

### **Conclusions**

Based on the results from this study, majority of the patients showed good glycemic control based n HbA1c compared to Glycated albumin. Random blood sugar showed no correlation with Glycated albumin or glycated hemoglobin. There was correlation between HbA1c and Glycated Albumin with  $R^2$  value of 0.64.

### ***Reccomendations***

The study found good correlation between HbA1c and Glycated Albumin which would support its utilization in monitoring glycemic control, however there is need for further studies to be done on characteristics of glycated albumin test and reference range validation in order to consider introducing it as a method of monitoring medium term glycemic control.



## **Introduction**

Diabetes Mellitus (DM) is metabolic disease that shares the phenotype of hyperglycemia. It is caused by a complex interaction of genetic and environmental factors. Hyperglycemia is caused by reduced insulin secretion, decreased glucose utilization and increased glucose production. Common symptoms include: polydipsia, polyuria and polyphagia. Weight loss and susceptibility to infections are common complications. Acute complications associated with DM include: diabetic keto-acidosis, hypoglycemia and hyperglycemic hyper-osmolar state. (1)

DM is classified on the basis of pathogenic process that leads to hyperglycemia. There are 2 broad categories, type 1 and 2. Type 1 is as a result of complete or near total insulin insufficiency which is due to autoimmune destruction of islet B cells or idiopathic causes it is associated with HLA: DQA and DQB genes. Type 2 is a heterogeneous group of disorders characterized by variable degree of insulin resistance, impaired insulin secretion and increased glucose production. It has a strong genetic predisposition in individuals with family history of the disease, individuals with hypertension and dyslipidaemias and in certain ethnic groups.

Maturity onset diabetes of the young (MODY) is a form of youth onset diabetes that is insulin – independent with a strong dominant family history and associated with abnormal hepatic nuclear factor and glucokinase genes. The other specific types of diabetes are gestational (onset during pregnancy), those associated with endocrinopathies, chemical and drug- induced, infections and genetic syndromes.

The global prevalence of DM has risen dramatically over the past two decades from 30 million in 1985 to 171 million today. Based on current trends more than 360 million people will have diabetes by the year 2030. Prevalence of type 2 is rising more rapidly because of increasing obesity and changing lifestyles as more people are leading sedentary lifestyles (2)

The number of diabetics in Africa is uncertain, but it is projected that the prevalence by the year 2030 there will be 18.9 million from 7 million in 2000 in the age bracket 20-79 years. In Kenya, the prevalence of diabetes is estimated to be 3.3%. This figure is based on regional

projections and is likely to be an underestimation as over 60% of people diagnosed to have diabetes in Kenya usually present to the health care facility to be managed for other illnesses. This raises an enormous healthcare concern as all African countries are already struggling to cope with diabetes burden. According to statistics Type 1 DM which used to be rare is now becoming more prevalent, this could be attributed to the fact that there is more aggressive and improved diagnostic programs for populations at risk with Diabetes Mellitus. Impaired glucose tolerance is becoming more problematic and exceeds 30% in many African countries (2)

Criteria for diagnosis of DM are: (i) Symptoms of DM and RBS of  $\geq 11.1$ mmol/l (ii) Fasting plasma glucose of  $\geq 7.0$ mmol/l (iii) 2hr plasma glucose of  $\geq 11.1$ mmol/l during an oral glucose tolerance test. Current criteria emphasize use of fasting plasma concentration as the most useful, reliable and convenient test for identifying DM in a symptomatic patient. There is an agreement by ADA and European association on Diabetes Mellitus study that HbA1c of  $>6.5\%$  can be used for diagnosis of DM. (3)

The progressive complications of unmanaged diabetes include heart disease, blindness, and kidney failure. Circulatory problems and nerve disorders lead to amputations. At diagnosis 25% already have retinopathy, 8% have nephropathy and 9% neuropathy.

Decades of research have established that prolonged exposure to excess glucose is the cause of diabetic complications and that long term control of blood glucose is required. The process of protein glycation is now understood to be both a marker for the progress of diabetes complications and underlying cause for many of the most serious complications (4)

Monitoring blood sugar levels in individuals with DM is currently done by a combination of short-term that is random blood sugar and long-term methods (glycated hemoglobin). Glycated albumin is an intermediate method for assessment of glycemic control which has been used in Japan and other Far East countries (5). HbA1c represents time averaged plasma glucose level of over 2-4 months; it requires longer time for HbA1c to improve after improvement of glycemic control as compared to GA whose time averaged plasma glucose level is 2-4 weeks. GA enables evaluation of treatment regimen faster.

American Diabetes Association describes the treatment goals for adults with DM as HbA1c of <7% (53mmol/mol), pre-prandial plasma glucose of 5.0-7.0mmol/l, peak post-prandial plasma glucose of <10mmol/l, Blood pressure (BP) of <130/80, LDL<2.6mmol/l, HDL >1.1mmol/l and Triglycerides of <1.7mmol/l. (6)

Kenyatta National Hospital attends to about 8,500 diabetic patients every year. Most of the patients are managed at the outpatient clinic while the others are hospitalized to receive inpatient care. Many of these patients are seen at long intervals (3-6months). Lately improvements have been made to enable diabetic patients with special individual needs to be reviewed more frequently by nurses and clinical officers at the clinic (7). Random blood sugar of >11.1mmol/l in symptomatic patients is used as a basis for diagnosis. Those with RBS of between 7.9mmo/l and 11.1mmol/l are subjected to oral glucose tolerance test to determine their diabetic status.

HbA1c is used in monitoring DM in patients in the private hospitals. The tests cost between USD15 and 20 USD which is far beyond the reach of many diabetic patients attending the Diabetic clinic at KNH. Glycated Albumin is a new useful and rapid method for monitoring DM. During the period of data collection, July to September 2011, HbA1c test was not being done at KNH labs but was introduced shortly later at subsidies prices of 11 USD.

## **Literature Review**

### **Blood glucose monitoring**

Blood glucose monitoring measures a point in time glucose concentration in blood. Self monitoring of blood sugar (SMBG) can only provide a snapshot of blood glucose levels and does not monitor glycation, hence has minimal benefit in improving glycemic control. The Fremantle study of 1,286 type 2 diabetes patients of over 5 years as well as a study of nearly 3,000 type 2 patients on oral medication or diet alone in Germany and Austria found no benefit for daily blood glucose testing regardless of treatment (8).

Another study done by Farmer in 2009 found little benefit for SMBG. In this study, type 2 patients with non-insulin treated diabetes were divided into three groups and followed for 12 months. All were given the same education as to how they could maintain or improve their condition: diet, exercise, etc. One group was given education and HbA1c testing every 3 months. The second group was given in addition a blood glucose meter, trained on its use, and told to test themselves 2 days a week and call a doctor if their results were above or below certain values. The third group was further given extensive training in using and interpreting the meter and encouraged to use it for multiple daily tests and to try to coordinate their lifestyle choices with meter results. After 12 months, the study found no significant improvement in glycemic control, for any group, in spite of setting conditions for the intensive group into a framework that, based on psychological theory, should have optimized its effect. (9)

Independent studies on glycemic control of DM have been done by three national organizations, the American Diabetes Association (ADA) American College of Endocrinology (ACE) and Veteran affairs Department of Defence (Va/DoD) each of these organizations state that SMBG is necessary for tight glycemic control. ADA recommends that it should be done three times or more for patients getting multiple insulin injections. The Va/DOD recommended that the SMBG alone does not improve glycemic control. The patient and medical health

providers need to review the glucose values recognize hyper and hypoglycemic episodes and attempt to correct by modifying the diet and exercise. (6)

### **Glycated hemoglobin**

There is evidence that optimization of glycemic control reduces the evidence of diabetes related complications of retinopathy and neuropathy (10) (11). In 1993 Otieno et.al assessed the quality of glycemic control on ambulatory diabetic patients attending the clinic at Kenyatta National Hospital and found out that more than 60% of the patients attending the clinic had poor glycemic control. They also found out that the group with poorest level of glycemic control was that on oral glucose lowering agents while those with the best control were those on diet only possibly because of fair endogenous insulin production. Poor glycemic control was presumed to be due to sub-optimal medication and deteriorating diabetes. (7)

HbA1c measurements have represented the gold standard for the evaluation of glycemic control in diabetic patient for the past 30 years. DCCT demonstrated that each 1% increase in HbA1c is associated with an increase in mean blood glucose concentrations of 2mmol/l and this increased the risk of progression and development of micro-vascular complications in DM (10)

HbA1c is most widely used to evaluate long term glycemic control (12) (10). In HbA1c circulating glucose combines with hemoglobin via a slow irreversible non enzymatic reaction determined by blood glucose concentrations ( (13) (14). Lower HbA1c levels are observed in individuals with decreased mean erythrocyte production rate. Hyperglycemia by itself is also known to reduce survival of erythrocytes. (12)

In general the methods available for measuring HbA1c fall into two categories: (i) Tests such as ion exchange chromatography and electrophoresis which rely on charge differences to separate the glucose, modified from the un-modified. Ion exchange chromatography is the most commonly used method for measuring HbA1c whether automated on High Performance Liquid Chromatography or run manually by mini- column procedures. (ii) The other methods include: immunoassay, calorimetric methods and affinity chromatography.

A number of caveats pertain to ion exchange and electrophoretic methods for HbA1c determination. These include potential interferences due to presence of elevated HbF, labile Schiff intermediates, lipemia, uremia and icterus. Hemoglobin variant carrying a negative charge may elute early causing a false estimation of HbA1c. In the case of unexpectedly high/low HbA1c levels the presence of an abnormal Hemoglobin variant should be sought and alternative method of measurement used. These interferences also occur with HPLC method used by DCCT. (10)

The American Diabetes Association recommends that HbA1c levels of <7% should be used for non pregnant individuals, less stringent HbA1c goals may be appropriate for patients with a history of severe hypoglycemia, limited life expectancy, advanced micro vascular and macro vascular complications, extensive co-morbid conditions and those with longstanding DM whose glycemic control is difficult to attain. For pregnant women HbA1c levels of 6.5% are recommended. (6)

The expert panel of National committee for Quality Assurance (NCQA) has recently developed new targets of HbA1c target of <8% they considered that target of <6.5% is difficult to achieve. The scoring was thereby revised for the updated standard so that the percentage of patients with acceptable levels HbA1c <8% are 40%. It is recommended that there be need to establish population specific cut-off thresholds according to ethnicity, age, gender and prevalence of DM.

The ADA recommends assessing HbA1c at least 2 times a year in patients who are meeting treatment goals and have stable glycemic control and quarterly in those patients whose hypoglycemic therapy has changed or those who are not meeting glycemic control goals.

In 2007, Bennet et.al published a systematic review of cross-sectional studies to assess the validity of HbA1c as a screening tool for early detection of DM. They concluded that HbA1c and FPG maybe equally effective as screening tools for DM. HbA1c thresholds of 6.1% was recommended .

In 2010 ADA finally included HbA1c value of  $\geq 6.5\%$  in the diagnostic criteria for diabetes mandating that the test be done in a laboratory using the NGSP (National Glyco-hemoglobin Standardization Programme) certified and standardized to the DCCT assay (6)

Current literature shows that there are areas of uncertainty in HbA1c testing due to biological variability, red blood cell variability and clinical variability. HbA1c values have been found to vary significantly within an individual and between individuals. Inter-individual variation is due to two components; those that are (i) glycemia related (ii) red cell variability. At the 67<sup>th</sup> ADA meeting in 2007, it was demonstrated that RBC variations in non anemic pre-menopausal women due to menstruation factors, low hemoglobin, hematocrit, MCV, MCH were negatively associated with glycemic control measured by HbA1c. In contrast, any RBC and iron metabolism indices were not associated with serum GA levels. These findings imply that HbA1c levels should be interpreted with caution when assessing premenopausal diabetic women and serum GA was suggested as a better index for chronic glycemic control in pre-menopausal women. (15)

It has been shown that 62% of the population variance in HbA1c level is genetically determined (16). It has also been proposed that HbA1c is only useful if erythrocyte turnover is not abnormal. High erythrocyte turnover is found especially in patients undergoing dialysis because erythrocyte lifespan changes due to periodic blood sampling, residual blood in the dialysis circuit, mechanical haemolysis, erythropoietin administration and blood transfusion.

Clinical variability arises from the broad disease spectrum encompassed by type 1 and 2 diabetics. A strong correlation has been demonstrated between fasting glucose and HbA1c for type 1, the correlation is considerably weaker in type 2. In a study by Jeffcoate et al, 2004 they concluded that there is doubt about the HbA1c as a predictor of micro-vascular disease in type 2 diabetes.

Correlation between A1C levels and mean plasma glucose levels based on data from the international A1C-Derived Average Glucose (ADAG) trial utilizing frequent SMBG and CGM in 507 adults (83% Caucasian) with type 1, type 2, and no diabetes. The American Diabetes Association and American Association of Clinical Chemists have determined that the correlation

( $r = 0.92$ ) is strong enough to justify reporting both an A1C result and an estimated average glucose (eAG) result when a clinician orders the A1C test

**Correlation of HbA1c with average glucose (6)**

HbA1c	6	7	8	9	10	11	12
Average glucose (mmol/l)	7	8.6	10.6	11.8	13.4	14.9	16.5
Average glucose (mg/dl)	126	154	183	212	240	269	298

The relationship between A1C and eAG is described by the formula:

$$\text{Averaged glucose ( mg/dl) } = 28.7 \times \text{A1C} - 46.7$$

$$\text{Averaged glucose (mmol/l) } = (28.7 \times \text{A1C} - 46.7) / 18$$

**Glycated Albumin**

Glycated Albumin has been reported as a rapid and useful indicator of glycemic control since the turnover of serum albumin is much shorter (half life of 17 days) than that of HbA1c.

Circulating albumin is strongly glycated at 4 sites of lysine residues and the glycation reaction occurs ten times more than in HbA1c. (17) (18). This implies that glycaemic fluctuation and excursion would influence glycation in albumin strongly.

Glycated albumin plays a dual role as indicator or marker of intermediate glycation and as a causative agent for diabetes complications. These complications include cardiovascular disease, Kidney failure, retinopathy and cognitive degeneration (Alzheimer’s disease). Extravascular protein accumulation leading to thickening of capillary basement membrane observed in micro vascular disease may be associated to protein glycation. A clinical research in China has further reinforced the linkage between levels of GA and coronary artery disease, leading the researchers to call for GA testing as a means of screening for Coronary artery disease. (19)



A study carried out by Abe et al , looking at glycemic control in diabetic patients on hemodialysis using glycated hemoglobin and glycated albumin, they found out that the two method were independently associated with blood glucose. However, HbA1c unlike GA was also influenced by hemodialysis, hemoglobin level and erythropoietin dose. Glycated albumin levels were not reflective in patients with massive proteinuria and those on peritoneal dialysis. They concluded that further studies needed to be done to confirm the target glycated albumin levels that are necessary to ensure good prognosis for patients with diabetes on dialysis. This means to set the cut off for good glycemic controls for diabetic patients on dialysis In addition more data was required to determine at which stage of kidney disease the measurement of GA would become more preferable to HbA1c. (20)

In a similar study by Barry I. et al looked at comparison of Glycated Albumin and HbA1c concentrations in diabetic subjects on peritoneal and hem dialysis. In this study, 470 diabetic patients on dialysis were recruited; out of these 212 were new patients. On the ne patients, HbA1c and %GA were run, it was found out that HbA1c were falsely low in patients with end stage renal disease weather they were on hemodialysis or peritoneal dialysis .The GA assays seemed to offer improved accuracy and not affected by dialysis. (21)

In a review article by Vernon et al on glycated albumin as an intermediate glycation index for controlling diabetes, the authors looked at the different methods of analyzing glyccated albumin and found out that 6 out of 50 labs in the united states perform GA testing and out of these 5 use affinity chromatography which gave them values in the ranges of 0.6-3% and the 6<sup>th</sup> lab used enzymatic assay and got reference range of 11-16%. Monoclonal isolation associated with ELISA as well as turbid metric and gel electrophoresis produced GA values in the lower range of reported values . The article also looked at the clinical utility of GA and noted that immediate applications are apparent for gestational and type 2 diabetes. This was supported by a clinical study in type 2 diabetics over 16 weeks which found out that GA decreased more rapidly than HbA1c as glycemic control improved. The ratio of GA to HbA1c was higher in hyperglycemic state than when diabetes was well controlled. (22)

Glycated Albumin has also been used for assessing for complications of diabetes. A study was done by Saba et. al which was looking at Value of Serum Glycated Albumin in Prediction of Coronary Artery Disease in Type 2 Diabetes Mellitus. Among the study population, 90 cases of with type II diabetes and undergoing diagnostic coronary angiography to find out the presence and extent of CAD were included in this study group and all individuals with type 1 diabetes rheumatoid arthritis and any other inflammatory diseases were excluded from the study.

The severity of CAD was based on lumen diameter narrowing as < 30 % (Minor CAD), 30-50% (mild CAD) 50-70 % (moderate CAD) and > 70 % (severe CAD) on visual assessment by experienced observer. Results showed that glycated albumin was more sensitive than glycosylated hemoglobin (HbA1c) in the evaluation of the severity of diabetes. The present study showed that glycated albumin was an independent risk factor for CAD in patients with type 2 diabetes, with and predicts the coronary artery disease in type 2 diabetes mellitus. (23)

One of the most reliable parameters of determining the relationship between two parameters is regression analysis, however the independent variable X is mathematically assumed to have none or very small errors when compared to dependent variable Y. Although HbA1c shows good proportionality to blood glucose, many patients show a discrepancy between values. HbA1c and GA levels are also proportional not only to plasma glucose levels but to the lifespan of RBC and half-life of albumin. (24)

In a study done by Yasuhiro et al, it was found out that HbA1c had better correlation with GA using the measurement error model (MEM) of linear regression analysis. This model deals with statistical analysis in cases where data for both x and y contain relatively large errors. The sources of these measurement errors include in HbA1c and GA derive from the fact that neither of the values accurately represent the mean plasma glucose level due to various factors affecting their plasma levels. There are assay errors, errors arising from differences in the delay time of HbA1c and GA after plasma glucose change, errors arising from inter-individual distribution of the lifespan of RBCS and half life of serum albumin and complications affecting them. (25)

## **Rationale**

Diabetes Mellitus is emerging rapidly as a major public health problem in developing countries both in numbers and costs of management. Studies done have cited the challenges of diabetes care to be economic incapacitation and lack of good laboratory support in units that operate in a low income environment like Kenyatta National Hospital.

The current diabetes monitoring paradigm consists of self monitoring blood glucose testing that does not measure glycation and HbA1c has inherent deficiencies despite it being the accepted method for long term monitoring of plasma glucose. However there is a demonstrable need for an intermediate glycation index. Measurement of GA monitors DM complications by showing damage to proteins over the previous 2-3 weeks. This closes the information gap that exists between daily blood glucose testing and HbA1c testing.

According to a study by Takahashi et al, it was shown that diabetes monitoring based on GA can reflect changes in treatment more quickly than other methods (26). It could be used as a monthly indicator for tight glycemic control and has immediate applications including testing for gestational DM.

In a period when rapidly escalating costs pose challenges in management of diabetic patients the glycated Albumin test stands out as a new approach , it has the potential to lower costs, increase patients compliance and serve as preventative function in response to one of the greatest challenges facing the world, the diabetes epidemic.

## **Research question**

Is there a comparison between Glycated Albumin and Glycated hemoglobin methods for monitoring glycemic control in Diabetes Mellitus patients at Kenyatta National Hospital?

## **Broad Objective:**

To compare glycated albumin to glycated hemoglobin levels as a measure of glycemic control for Diabetic patients attending outpatient clinic at Kenyatta National Hospital.

### **Specific Objectives:**

1. To determine random blood glucose in both type 1 and type 2 diabetic patients.
2. To determine levels of glycated albumin and glycated hemoglobin in study population.
3. To correlate the glycated albumin and glycated hemoglobin levels in study population. The levels of glycated hemoglobin and glycated albumin were also compared to the type of diabetes, type of treatment, duration of diabetes, body mass index and random blood sugar levels.

### **Materials and Methods**

#### ***Study design***

Comparative cross-sectional descriptive study.

#### ***Study Areas***

Diabetic clinic at Kenyatta National Hospital. The main clinic for diabetic patients is held on Fridays where patients are seen once a year and the mini clinics run from Monday to Thursday. The total number of patients, both new and old seen at the clinic yearly are between 8,000 and 9,000 in number and out of these new cases are about 600.

Glycated hemoglobin was estimated in the Thematic area of Clinical Chemistry laboratory, Department of Human Pathology, University of Nairobi. Glycated albumin was run and KNH lab 16.

#### ***Study population***

The study population consisted of diabetic patients attending the diabetic clinic at Kenyatta National Hospital from the period of July to September, 2011. The study subjects were recruited daily from Monday to Friday whether they were attending the minor or major clinics.

The parameters used to describe good diabetic control were: HbA1c of less than 7% (53mmol/mol), glycated Albumin of less than 285umol/l and the parameters for random blood glucose were assessed using the glucose control chart used at the clinic developed by Diabetes association of Kenya for use in Kenyan public hospitals.

***Inclusion criteria were:***

1. Patients seen at the diabetic clinic
2. Both type 1 and type2 diabetic patients
3. Both male and female gender
4. Those who gave informed consent to participate in the study

***Exclusion criteria***

Those with confirmed /documented Diabetic complications

***Sample size***

Fisher's formula

$$n = \frac{Z^2 \times P(1-P)}{d^2}$$

Where

n = desired sample size in an infinite population

z = standard normal deviate - 1.96

p = proportion of the characteristic of interest – estimated proportion of patients with poorly controlled blood glucose; 60% from previous studies (Otieno C.F et al ,EMJ,2003 )

q = (1 – p) =40%

d = the degree of accuracy set at 0.05.

Therefore the minimum estimated sample size is

$$= \frac{1.96^2 \times 0.6 \times 0.4}{0.05^2} = 369$$

Since this study has a finite population (less than 10,000), an adjustment was done using

$$n_f = \frac{n}{1 + n/N} \quad 16$$

Where:  $n_f$  – adjusted sample size  
 $n$  – Calculated sample size for a finite population =369  
 $N$  – The estimated total population in which the sample will be drawn; estimated at 8,500 per year, 875 in 3 months,  
 $n_f = 260$  (27)

### **Case definition**

According to ADA good glycemic control is defined as:

HbA1c of <7% (53mmol/mol), pre-prandial plasma glucose of 5.0-7.0mmol/L, peak post-prandial plasma glucose of <10 mmol/L There is no definition for Glycated Albumin given hence cut off of 285umol/l was used based on the parameters from the Diazyme Kit assay (6)

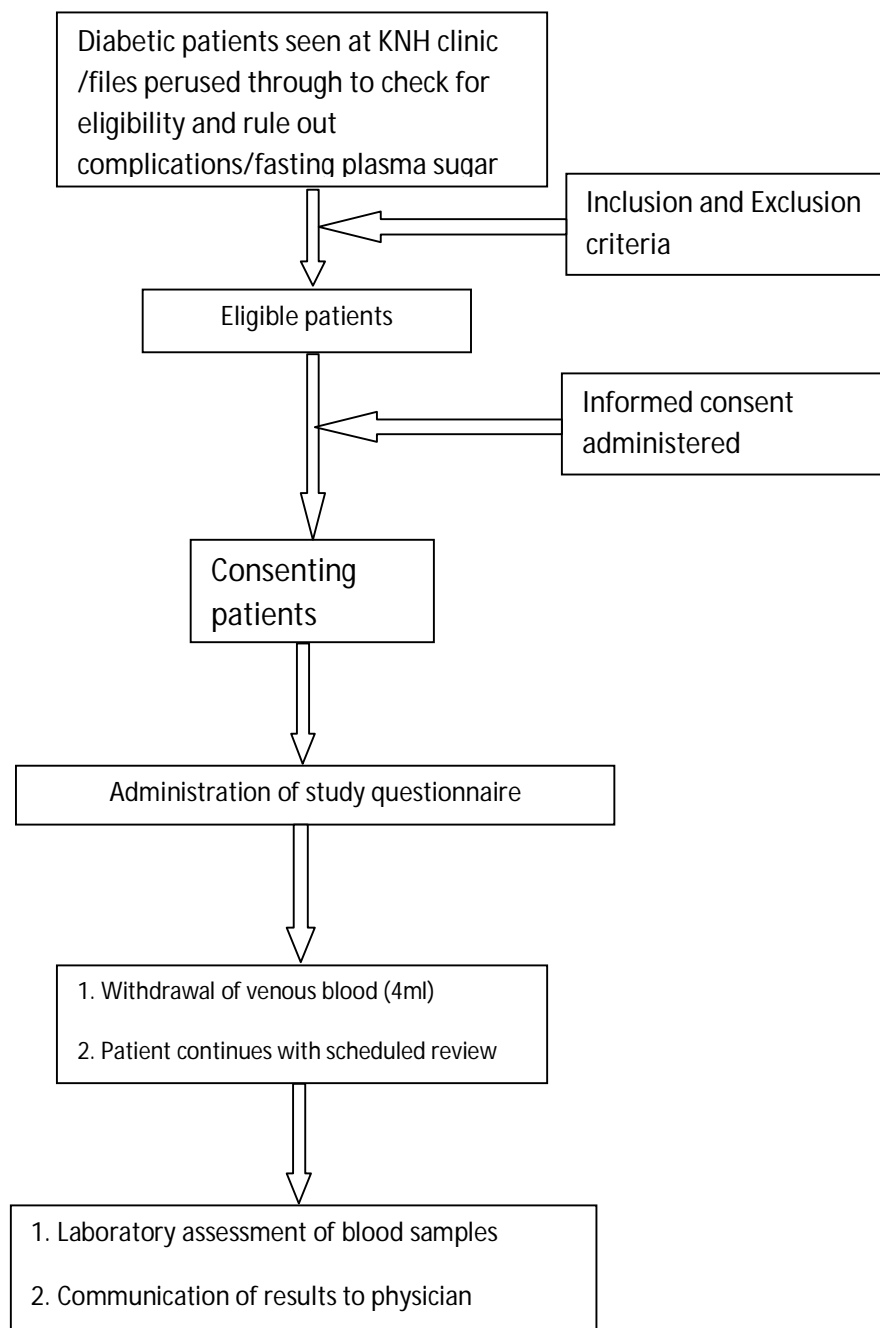
### ***Sampling and Data collection procedure***

Diabetic patients attending the clinic were assessed for eligibility and recruitment done consecutively until the desired number was attained. This task was carried out by the principal investigator. Screening was done by perusing the files to check for potential study participants who fit the inclusion criteria and those who did not have documented diabetic complications.

To participate in the study, the participants were briefed on the objectives of the study (Appendix I) and those who agreed to participate were consented by the principal investigator (Appendix II). The assent form was not filled because among the patients recruited none was below 20yr of age. The patients who are seen in the diabetic clinic are those above 14 yrs, however during the study period none of the patients were below 20yrs.

The study questionnaires (Appendix III) were administered by the Principal investigator, random blood sugar recorded and assessed for glycemic control using the ADA guidelines. Body mass index were then calculated after the heights and weights were taken. Blood was drawn by a trained research assistant from all the interviewed patients for laboratory analysis of GA and HbA1c.

**Figure1:** Flow chart for sampling and data collection procedure



## **Laboratory Procedures**

### ***Specimen collection, analysis and storage and quality control***

After the questionnaires were filled by consenting patients, the patients were seated in a procedure room and the process of blood sampling explained to each one of them by the primary investigator and the research assistant.

The site for venepuncture was cleaned with swabs soaked in 70% alcohol and dried using a dry cotton wool. Approximately 4 ml of blood was drawn from the cubital vein of each participant using a sterile needle and syringe. The sample was then divided into two: 2ml was put in the EDTA bottles and sample mixed well with anticoagulant to avoid clotting of sample. The other 2ml was put in a plain bottle.

The EDTA anticoagulated blood was used to run the glycated hemoglobin which was done within 4 hrs of sample collection. The samples in the plain bottle were centrifuged after the daily collection and stored in cryo vials at -20°C. The samples were later run as a batch for analysis of glycated albumin .

To ensure quality and reliability of results, venous blood was collected using aseptic technique. Proper volumes (4ml) of blood was collected and mixed adequately, Clotted samples were discarded.

### ***Role of the Principal investigator***

The PI was involved in recruitment of the study subjects and perused through their files to exclude those with diabetic complications. After that, consent was filled by the patients willing to participate in the study after the objectives were explained to them. In collaboration with the research assistant the PI collected the blood samples for analysis.

The PI was also involved in running of the samples especially the HbA1 where calculations to derive HbA1 are involved, and also making sure that controls were run and were within reference ranges before running of the test samples.



## **Analysis of samples**

The Hemoglobin, random blood glucose, Glycated hemoglobin and Glycated albumin were initially to be done at molecular and diagnostics laboratory, KNH but the machine broke down two weeks before data collection.

## **Analysis of Blood Glucose**

This was done using a glucometer at lab 16 or at the clinic. The patients who were recruited from the minor clinics usually pass through lab 16, KNH to have their blood sugars taken. The patients who were recruited from the major clinic had their RBS done at the clinic by the technician from the lab 16.

### Principle of test

It adopts an electrochemical detection method and dry reagent strip technology. A drop of whole blood is placed on the tip of the strip, it is automatically drawn into the reaction chamber through capillary action and a reading is displayed on the meter. The linearity range is 1.1-33.3mmol/l

### Quality control

Test strip is packaged in a cool dry place, not exposed to sun and not refrigerated.

Strips are handled with dry hands.

Each strip was used immediately after removing from the vial and was used once.

## **Analysis of Hemoglobin**

This was done at Molecular and diagnostics laboratory KNH. Sample was run within 4 hrs after collection into an EDTA anti-coagulated blood. This was done using Cell Dyn 1700.

### Principle

The principle used for hemoglobin estimation is modified cyanmethemoglobin method.

## Quality control

Commercially prepared trilevel controls representing normal, low abnormal and high abnormal are used for internal quality control to check for accuracy before running samples.

Reference ranges that are age and sex adjusted was used in the machine.

The laboratory participates in EQA (HUQAS) quarterly

## **Analysis of HbA1c**

Analysis of HbA1 was done in the Clinical Chemistry Unit, University of Nairobi using Humalyser 2000, which is a semi-automated machine using the principle of spectrophotometry.

## Principle of the Test

Glycated HbA1 was analysed according to the method of fast ion-exchange resin separation method using kits provided by HUMAN, Human Gesellschaft für Biochemica und Diagnostica mbH, Germany. Whole blood is mixed with a lysing reagent containing a detergent and borate ions. Elimination of labile Schiff's base is thus achieved during the hemolysis. The hemolyte is then mixed for 5 minutes with a weakly binding exchange resin. During this time the HbA1c binds to the resin. A special resin separator is used to remove the resin from the supernatant fluid which contains the HbA1. The glycohemoglobin percentage of total hemoglobin is determined by measuring the absorbance of the glycohemoglobin and of the total hemoglobin fraction at 415nm in comparison with a standard glycohemoglobin preparation carried through the test procedure.

## Procedure

The analysis was done by the PI assisted by the technologists at the Unit.

1. Preparation of hemolyte
2. HbA1c determination

$\% \text{HbA1} = \text{HbA1 sample} / \text{total hemoglobin} \times \text{factor}$

Estimate of HbA1c was made using the formula  $\text{HbA1c} = 0.9 \text{HbA1} + 0.05$ . (28)

### Quality Assurance

Whole blood was used with EDTA as anticoagulant and test run within 4 hrs

The reagent, lysing agent and standard were stored at 2-8<sup>0</sup> c

The internal controls were run daily before running of the test samples.

The lab participates in EQA with HUQAS for other parameters but not HbA1

The chemistry labs in the university where these samples were run participate in EQA (HUQAS) quarterly.

### **Analysis of Glycated Albumin**

The analysis was done at KNH chemistry laboratories (lab 16) using the Olympus AU 640.

The serum stored at stored in cryo vials at 0-20<sup>0</sup>C then were run as a batch and results tabulated.

### Assay principle

Glycated Albumin was estimated using a kit from Diazyme, California USA. The Diazyme Glycated Serum Protein Assay uses protein K to digest GSP into low molecular weight fragments. The hydrogen peroxide released is measured by a calorimetric Trinder end-point reaction. The absorbance of 600nm is proportional to the concentration of glycated serum proteins.

The reagents 1 and 2 are ready for use, 20ul of sample was used.

The turn around time for running the test is 10 mins.

Adults (20-60 yrs) have a reference range of 100-28

### Quality Assurance

The reagents were stored at 2-8<sup>0</sup> C.

Calibration of the analyzer was done before running of the test.

The internal controls supplied by the manufacturer were run before the test samples were run.

Further quality control measures taken to ensure reproducibility of results included maintenance of analyzers. The equipments usually undergo daily and weekly user maintenance, they also have scheduled preventive maintenance every 3 to 6 months.

The KNH lab participates in EQA (HUQAS) for other analytes not glycated Albumin because it was being run for the first time.

### **Data management and analysis**

Data was entered and managed into Microsoft Access database. Data analysis was performed using SPSS version 17 software. The demographic characteristics of the patients such as age and gender were summarized using means and proportions where appropriate. Laboratory parameters were also summarized using means or medians and proportions.

Using the HbA1c as the gold standard test, the patients were categorized into two groups: normal HbA1c less than 7% and high HbA1c (more than or equal to 7%), these two categories were used to describe good and poor glycaemic controls. Then the two groups were compared according to the demographic characteristics. Chi square and student t-tests were used when comparing categorical and continuous variables respectively.

HbA1c was compared with GA using linear correlation graphs. The cut off for glycated albumin was 285umol/l, those with levels below were described as having good glycaemic control while those with levels equal to or above 285umol/l were described as having poor glycaemic control.

All comparison tests were performed at 5% level of significance and 95% confidence interval. Random blood sugar levels were compared to the GA and HbA1c.

### **Ethical Considerations**

The study was carried out after approval was obtained from the Department of Human Pathology and KNH/UoN Ethical and Research Committee.

1. All participants in the study were informed that their participation in the study was totally voluntary and no remuneration was offered.
2. A consent was signed which briefly defined the study and its importance.
3. All the information obtained from the participants was treated with confidentiality.
4. Assurance on access to the results and their medical interpretations was assured.
5. Appropriate referrals were made for medical intervention.

### **Limitations of study**

1. Effects of hypo-albuminea and hyper-albuminea were not determined in this study

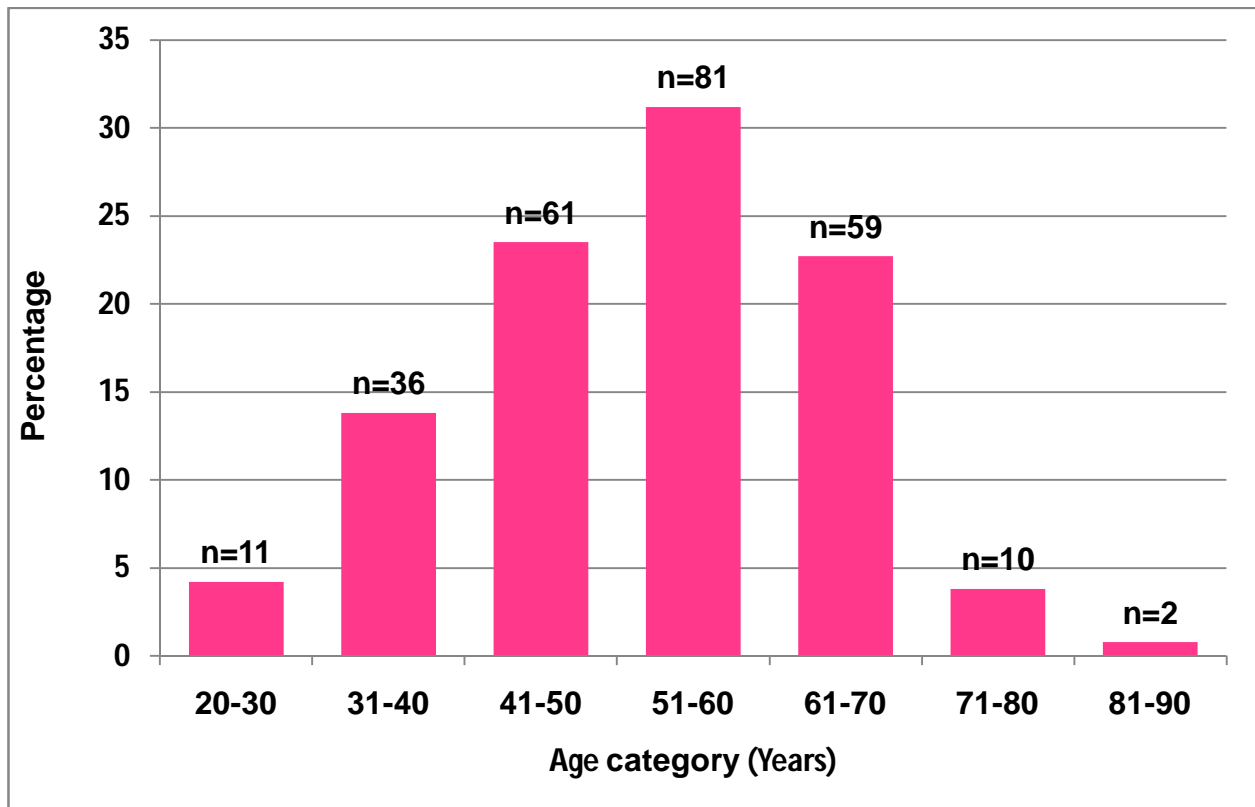
## **RESULTS**

The data collection was carried out between July and September 2011. During this period, 272 files were scrutinized. After perusing the files, 5 were found to have some complications especially deranged renal function tests and the other 7 declined to consent. Afterwards, 260 patients were recruited into the study.

Socio-demographic data of the study population

A total of 260 patients met the eligibility criteria and formed analysis for the study.

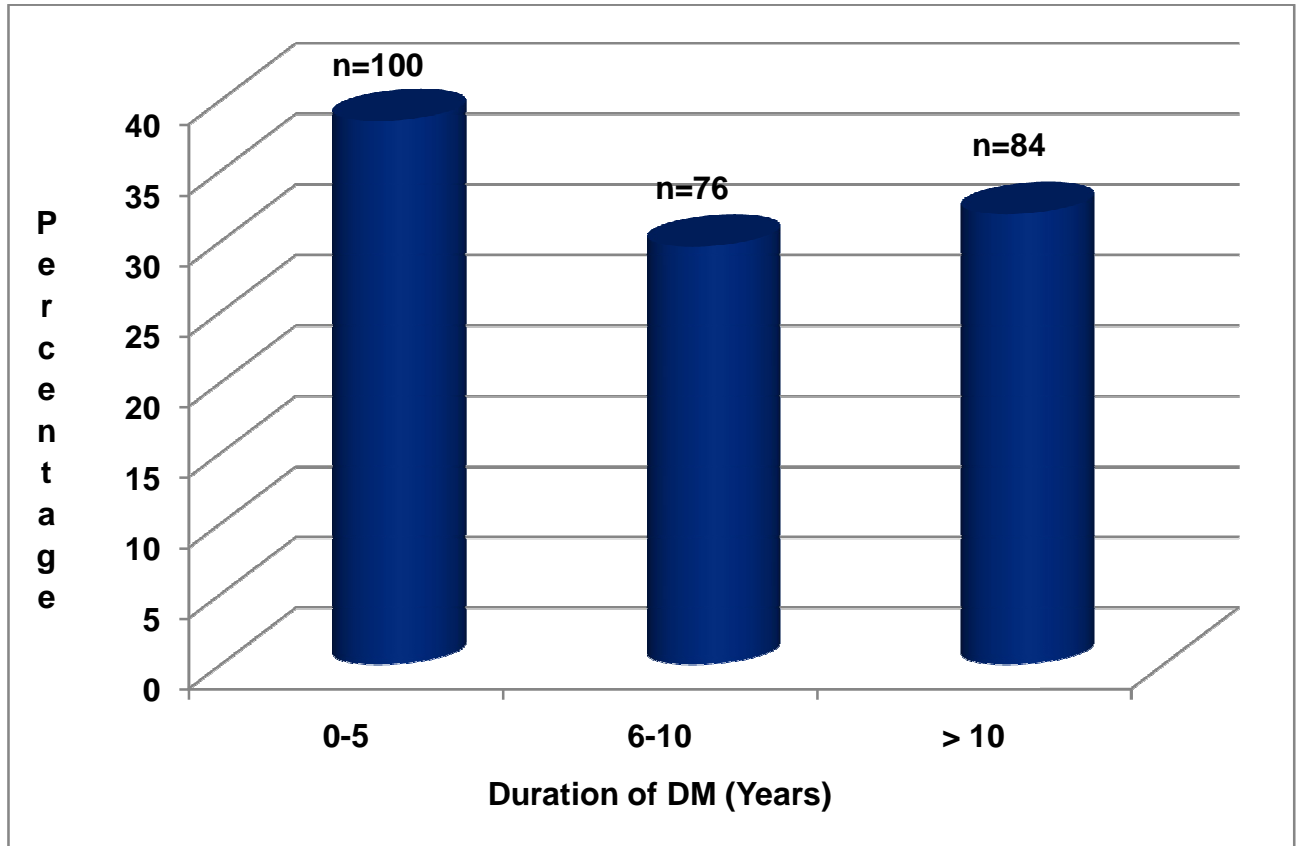
**Figure 2: Age distribution (n=260)**



The youngest person was 20 yrs old and the oldest was 90 yrs old. The mean age was 52.5yrs, with a standard deviation of 12.3. Majority of the participants were in the 51-60 yrs. This group had 81 (31.2%) diabetic patients attending the out patient clinic. There were only 2(0.8%) patients who were above 81 yrs. The 20-30yr olds constituted 4.2% of population while the 31-40 yr olds were 36 (13.8%) (Figure 2)

There were 157 (60.4%) females and 103 (30.6%) males.

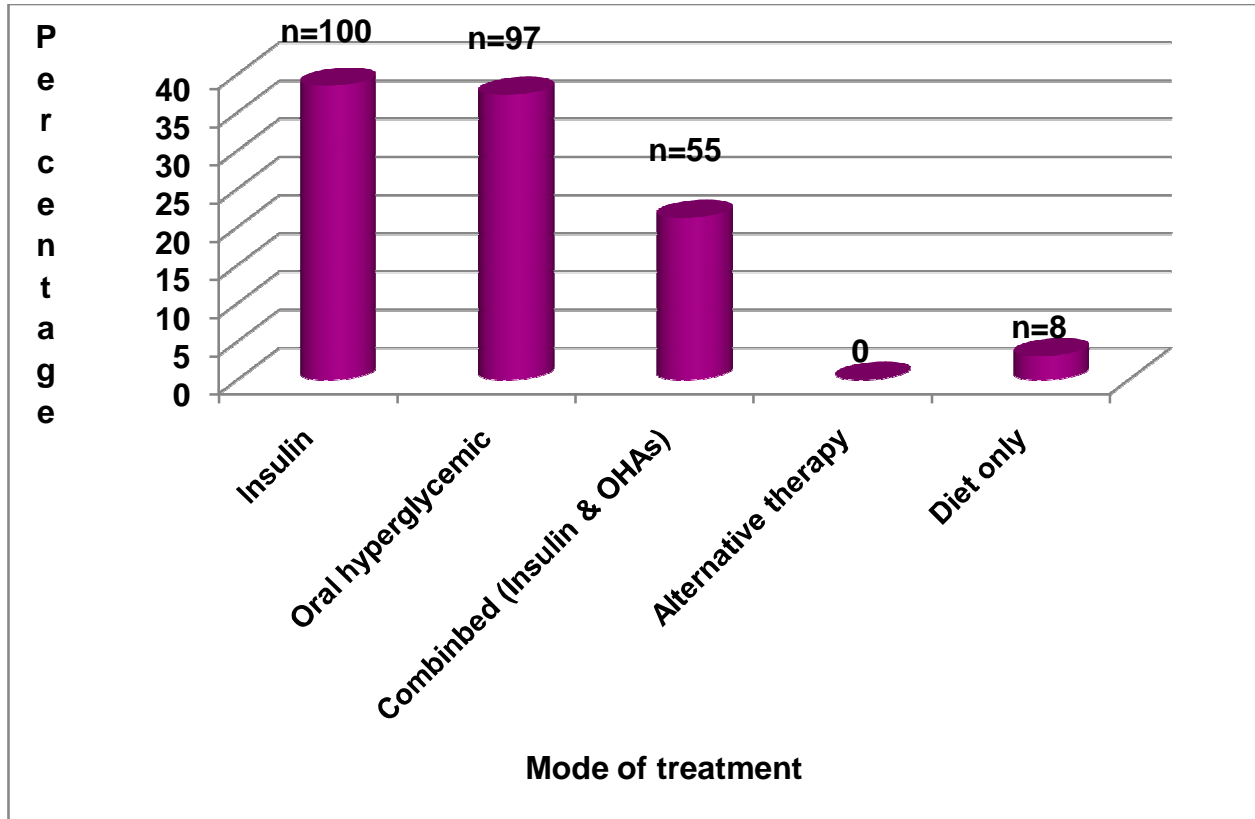
Figure 3: Duration of Diabetes Mellitus since diagnosis (n=260)



Most of the patients were relatively newly diagnosed with 0-5 yrs since onset of the disease. This group had 100 (38.5%). The ones who had the disease for 6-10 yrs had 77 (29.6%) and those who had the disease for more than 10 yrs formed 83 (31.9%) (Figure 3)

Majority of the patients had type 2 diabetes, 214 (82.3%), with only 46 (17.7%) being type 1.

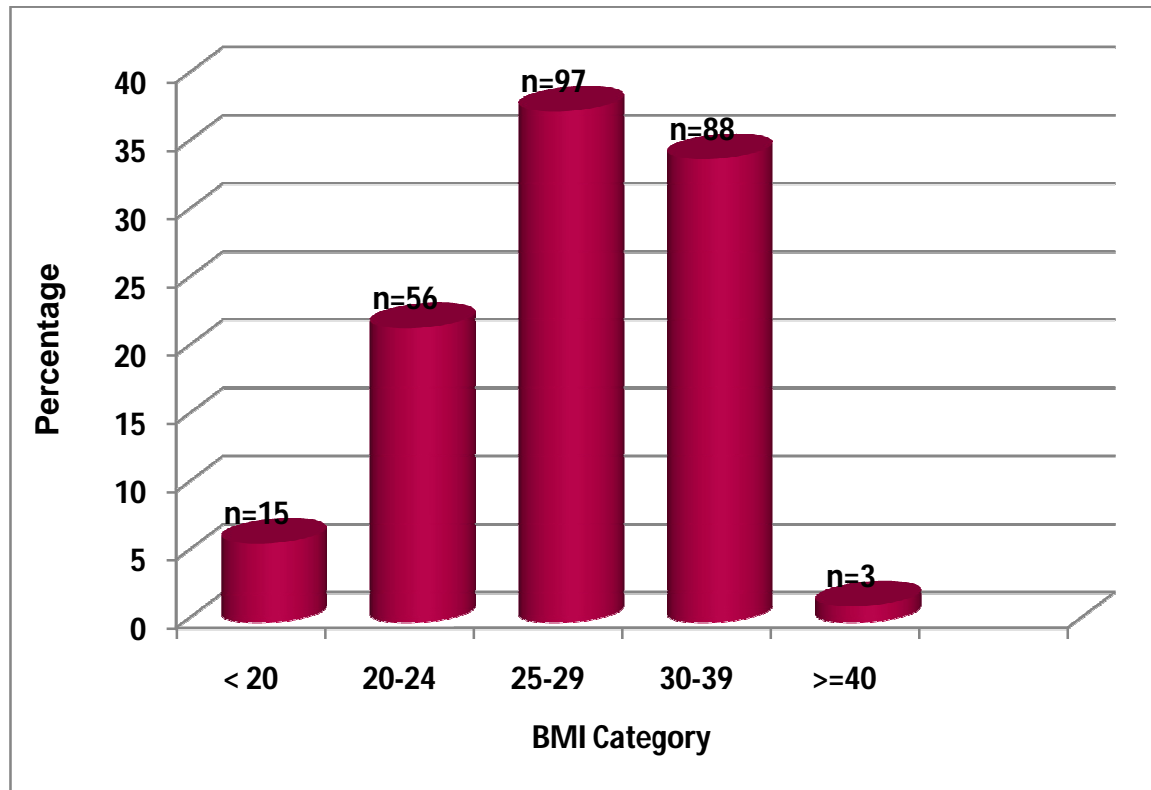
Figure 4: Type of treatment used by the diabetic patients (n=260)



Insulin was the main type of treatment used by the study participants. There were 100 (38.5%) patients who were on insulin. Those on Oral glucose lowering agents were 97 (37.3%) and those on combined therapy (insulin and OHA were 55 (21.2%). Only 8 (3.1%) were not on any medication. There was no patient on alternative therapy (Figure 4)



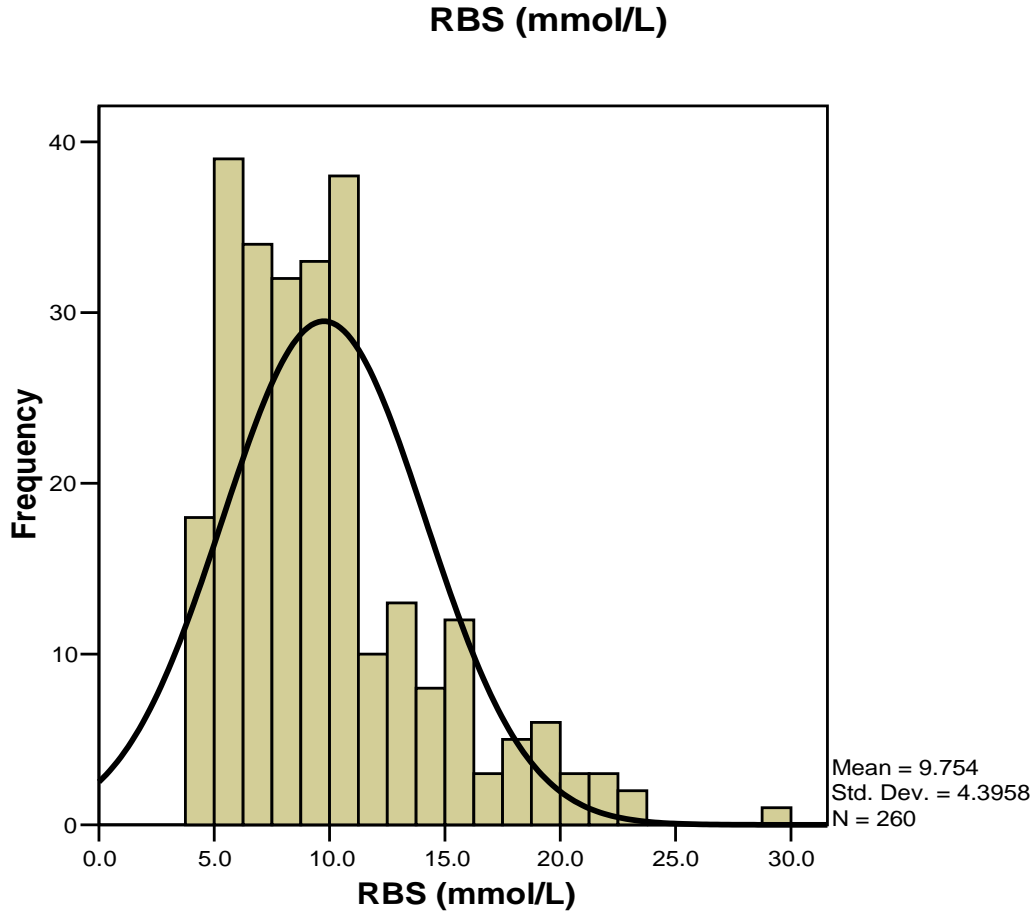
Figure 5: Body Mass Index of diabetic patients at KNH (n=260)



The lowest BMI in the study population was 16.5 while the highest was 42. The mean was 27.3 with a standard deviation of 5.

Most of the patients 97 (37.5%) were in the overweight category, followed by the obese patients 88 (34%). Only 3 patients were very obese. The ones who had ideal BMI that is a BMI of 20-29 were 56 (21.6%) and only 15 were underweight (figure 5)

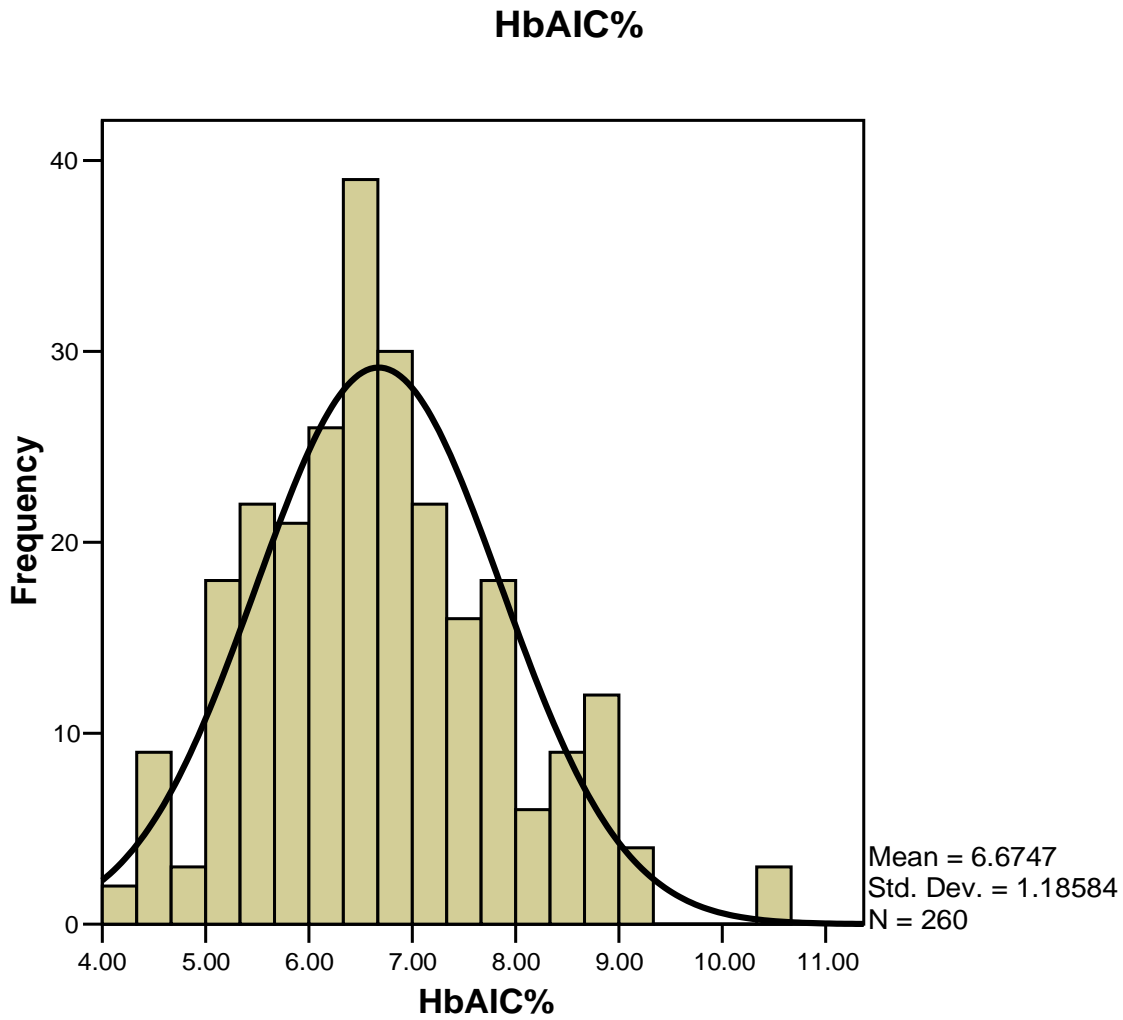
Figure 6: Histogram showing distribution of Random blood sugar in mmol/L in the study population. (n=260)



The range of the random blood sugar is 3.9-29.0mmol/L. The mean is 9.75 and the SD is 4.39. In majority of patients the random blood sugars was less than 10mmol/L which was considered as good glycemic control. (Figure 6)

Random blood sugar analysis showed that, the population with good glycemic control constituted 156 (60%).

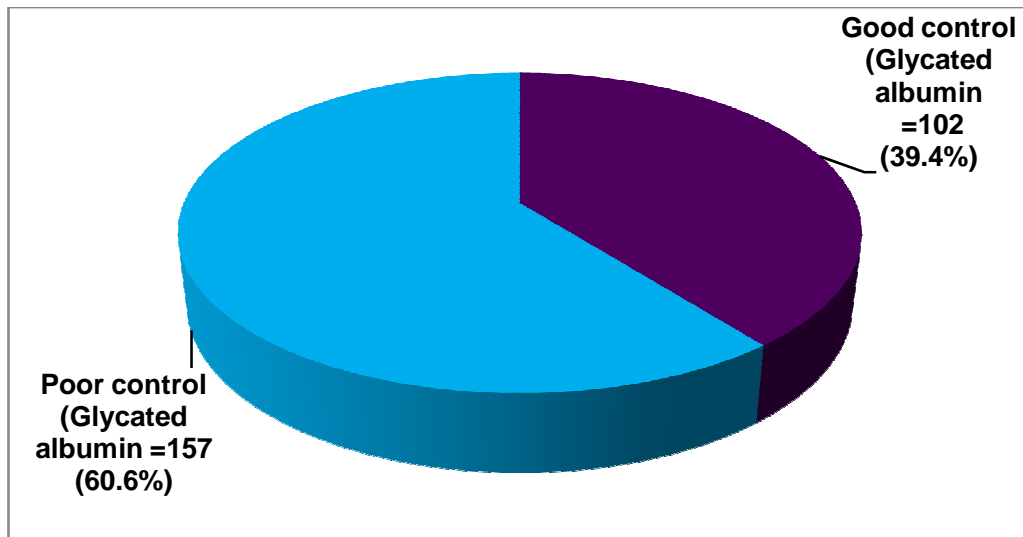
**Figure 7: Histogram showing distribution of Glycated Hemoglobin levels in the study population. (n=260)**



The histogram shows the distribution of Glycated hemoglobin level in the study population. The range was 4.1-10.8 and the mean was 6.67 with a standard deviation of 1.18. The patients with good glycemic (<7%) control constituted 65.4% of the study population. (Figure 7)

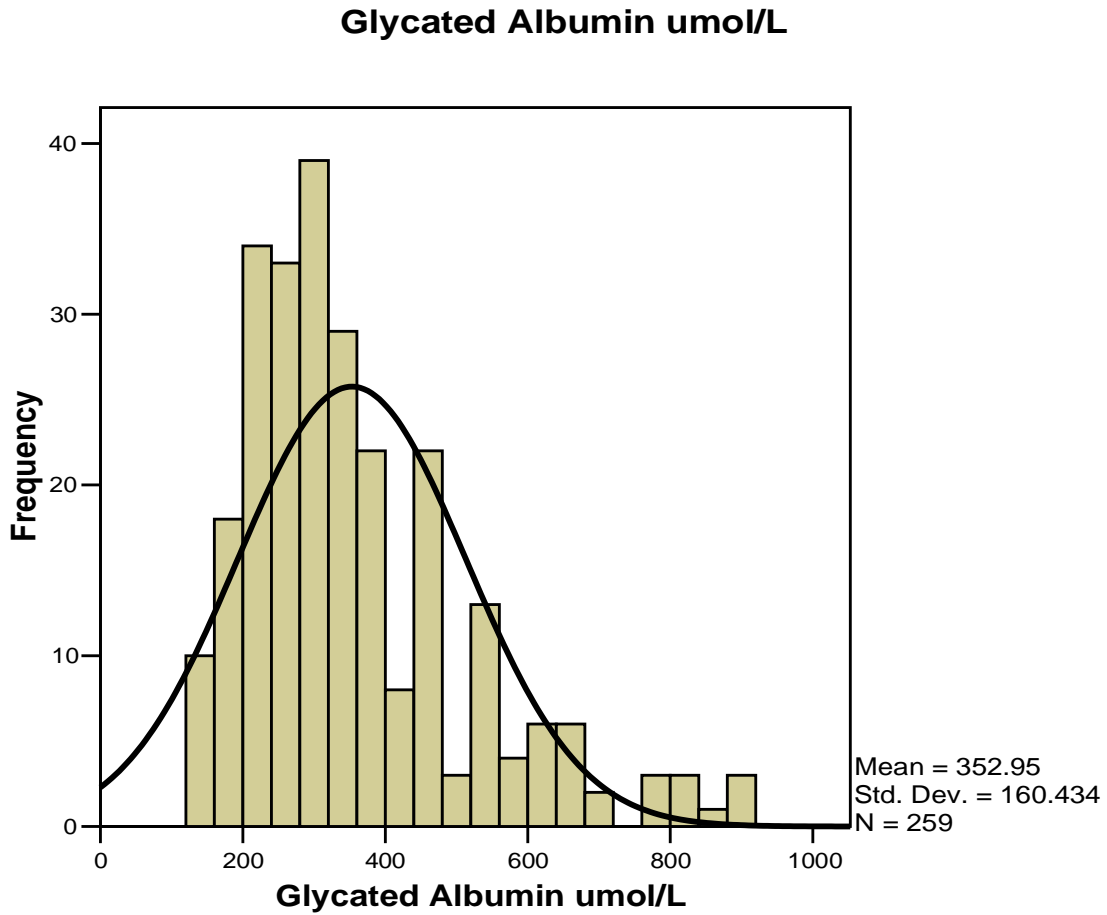
Results from this study showed that majority of the patients had good glycemic control 170 (65.4%) based on the HbA1c assay

**Figure 8: Glycated Albumin levels in the study population grouped into good (<285umol/l) and poor control (> or =285umol/l) (n=260)**



Majority of patients 157 (60.6%) had poor glycemic control while 102(39.4%) had good glycemic control (Figure 8).

Figure 9: Histogram showing distribution of Glycated Albumin in the population.(n=260)



The range is 120-920umol/L. The mean is 352.95 and the SD is 160.4 .(Figure 9). As shown in the histogram there were more patients with poor glycemic control (more or equal to 285umol/L).

**Table 1: Correlation between Glycated hemoglobin and Type of DM (n=260)**

	Glycatedhemoglobin (%)		Total	
	< 7 (Good Control)	(>=7 (Poor ontrol)		
Type of DM	1	28(60.9)	18(39.1)	46 (100)
	2	142(66.4)	72(33.6)	214 (100)
Total		170(65.4)	90(34.6)	260 (100)

A higher percentage of patients in type 2 (66.4%) had good glycemic control compared to type 1 (60.9%). There were only 18 (39.1%) of the type 1 who had poor glycemic control compared to 72 (33.6%) in type 2. Fisher's Exact Test showed that there was no association between glycemic control and type of Diabetes p value is 0.33 (Table 1).

**Table 2: Correlation between Glycated hemoglobin and duration of DM since diagnosis (n=260)**

		Glycated hemoglobin (%)		Total
		1. < 7 (Good Control)	2. (>=7 (Poor control)	
Duration of DM	0-5yrs	31 (31.0)	69 (69.0)	100 (100)
	6-10yrs	33 (42.9)	44 (57.1)	77 (100)
	>10yrs	37 (44.6)	46 (55.4)	83 (100)
Total		101 (38.8)	159 (61.2)	260 (100)

The patients who had longer duration of the disease were better controlled. Among those who had the disease for >10yrs, 37 (44.6%) had good glycemic control, while those who had the disease for 0-5yrs, 31 (31%) had good glycemic control. The ones who had the disease for 6-10 yrs 44 (57.1%) had good glycemic control. There was no association between glycemic control and the duration of Diabetes p value is 0.12 (Table 2).

**Table 3: Correlation between Glycated Hemoglobin and Type of Treatment (n=260)**

		Glycated hemoglobin (%)		Total
		1. < 7 (Good Control)	2. (>=7 (Poor control)	
Treatment	Insulin	61(61.0)	39(39.0)	100 (100)
	OGLA	73(75.4)	24(24.7)	97 (100)
	Insulin and OGLA	29(52.7)	26(47.3)	55 (100)
	Diet only	7(87.5)	1 (25.0)	8 (100)
Total		170 (38.8)	90 (61.2)	260 (100)

There were 61 (61%) patients on insulin who had good glycemic control and 73(75.4%) on oral glucose lowering agents. Only 29 (52.7%) on combined therapy (insulin and OGLA) had good control. As for the patients on diet, 87.5% had good glycemic control. Pearson chi-square was 10.649 and there was a strong association between glycemic control and type of treatment p value is 0.001 (Table 3).



**Table 4: Correlation between Categorised BMI and Glycated hemoglobin(n=260)**

		Glycated hemoglobin (%)		Total
		< 7 (Good ontrol)	>=7 (Poor ontrol)	
BMI category	< 20	10 (66.7)	5 (33.3)	15 (100)
	20-24	42 (75)	14 (25)	56 (100)
	25-29	65 (67)	33 (33.0)	98 (100)
	30-39	52 (59.1)	36 (40.9)	88 (100)
	>=40	0 (0)	3 (100)	3 (100)
Total		100 (38.6)	160 (61.4)	260 (100)

Amongst the patients with ideal BMI (20-24), 42 of them that is (75%) had good glycemic control while only 10(66.7%) in the underweight category had good control. All the patients with BMI of >40 had poor glycemic control. There was no association between BMI and glycemic control, p value is 0.12 (Table 4).

**Table 5: Correlation between Glycated albumin and type of DM (n=260)**

	Glycated albumin (%)		Total	
	< 7 (Good Control)	(≥7 (Poor control)		
Type of DM	1	15 ( 32.6)	31 (67.4)	46 (100)
	2	87 (40.7)	127 (59.3)	214 (100)
Total		102 (39.4)	158 (60.6)	260 (100)

Amongst type 1 diabetics, 15 (32.6%) of them had good glycemic control while in the type 2, 87 (40.7%) had good control. There was no association between glycemic control and type of DM, p value is 0.32 (Table 5).

**Table 6: Correlation between Glycated Albumin and duration of Diabetes Mellitus(n=260)**

		Glycated albumin (%)		Total
		1. < 7 (Good Control)	2. (>=7 (Poor control)	
Duration of DM	0-5yrs	31 (31.0)	69 (69.0)	100 (100)
	6-10yrs	34 (44.7)	43 (55.3)	77 (100)
	>10yrs	37 (44.6)	46 (55.4)	83 (100)
Total		102 (39.4)	158 (60.62)	260 (100)

The patients with longer duration of diabetes were found to have better glycemic control 37 (44.6%) compared to those with shorter duration 31(31%). Statistically there was no association between Glycated Albumin and duration of DM, p value is 0.09 (Table 6).

**Table 7: Correlation between Glycated Albumin and Type of Treatment(n=260)**

		Glycated albumin (%)		Total
		1. < 7 (Good Control)	2. (>=7 (Poor control)	
Treatment	Insulin	42 (42.0)	58 (58.0)	100 (100)
	OGLA	43 (44.8)	54(55.2)	97 (100)
	Insulin and OGLA	11 (20.0)	44 (80.0)	55 (100)
	Diet only	6 (75.0)	2 (25.0)	8 (100)
Total		102 (39.4)	158 (60.6)	260 (100)

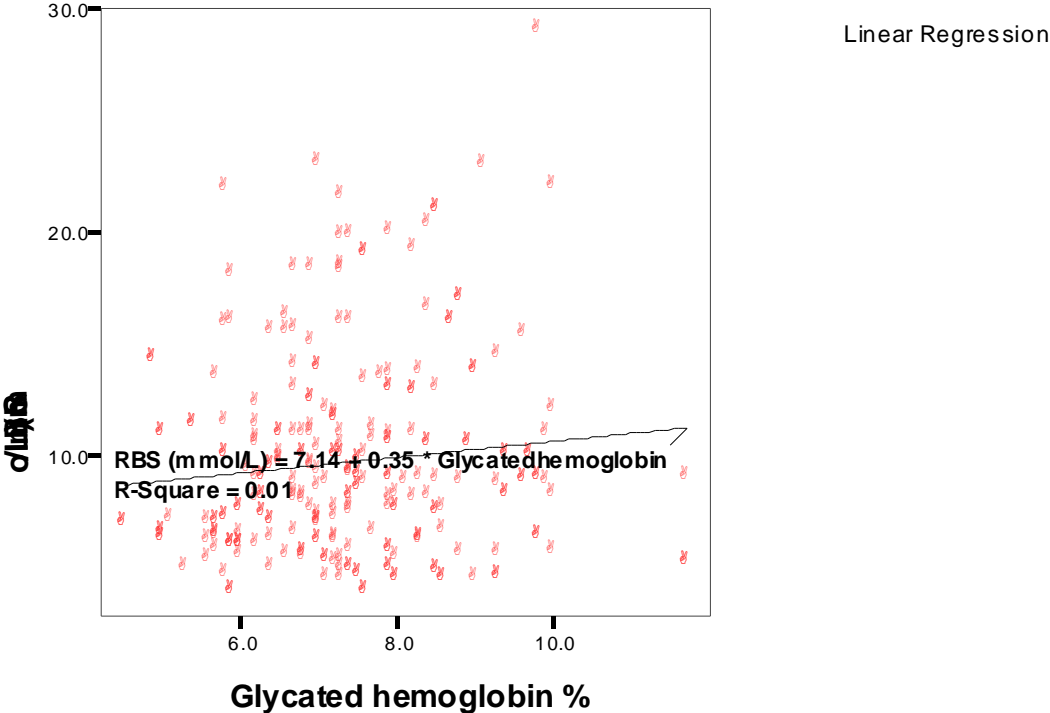
The patients with high percentage of poor glycemic control 44 (80%) were the ones on combined therapy (insulin and oral glucose lowering agents). There is a strong association between glycemic control and type of treatment, p value is 0.002 (Table 7).

**Table 8: Correlation between Categorised BMI and Glycated albumin(n=260)**

		Glycated albumin (%)		Total
		< 7 (Good ontrol)	>=7 (Poor ontrol)	
BMI category	< 20	6 (33.3)	10 (86.7)	16 (100)
	20-24	27 (48.2)	29 (51.8)	56 (100)
	25-29	37 (38.1)	61 (61.9)	98 (100)
	30-39	32 (36.8)	55 (63.2)	87 (100)
	>=40	0 (0)	3 (100)	3 (100)
Total		102 (39.1)	158 (60.9)	260 (100)

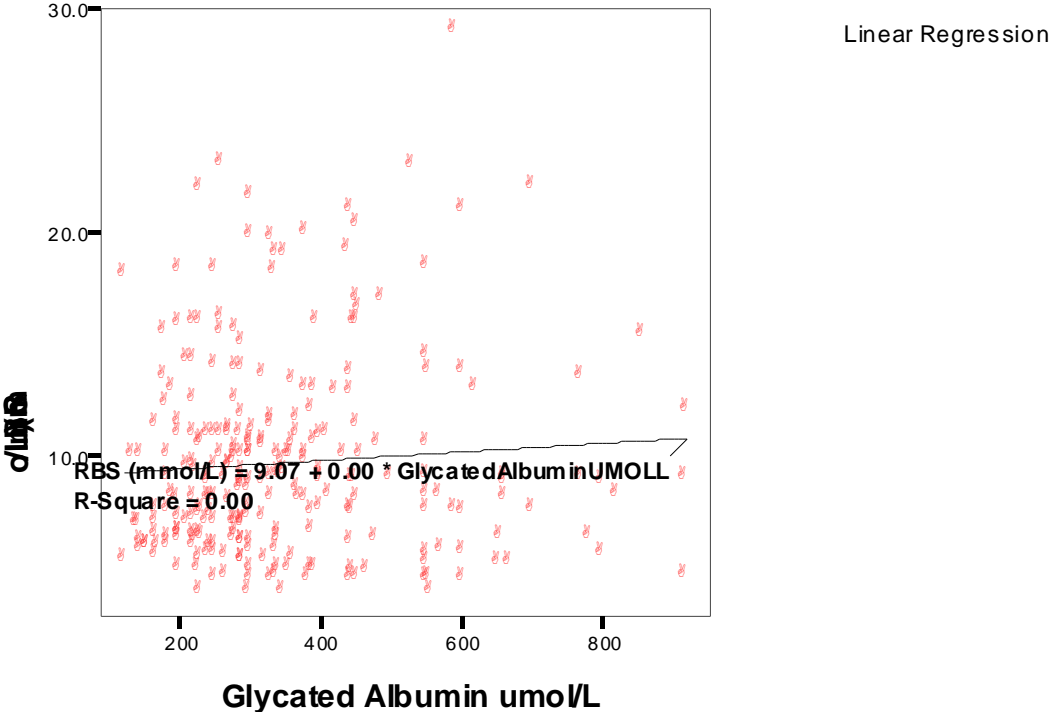
Patients with ideal body weight had better glycemic control were 27 (48.2%) compared to those who were overweight 37(38.1%). There was no association between glycemic control and BMI, p value is 0.36 (Table 8).

Figure 10: Correlation between Random Blood Sugar and Glycated hemoglobin



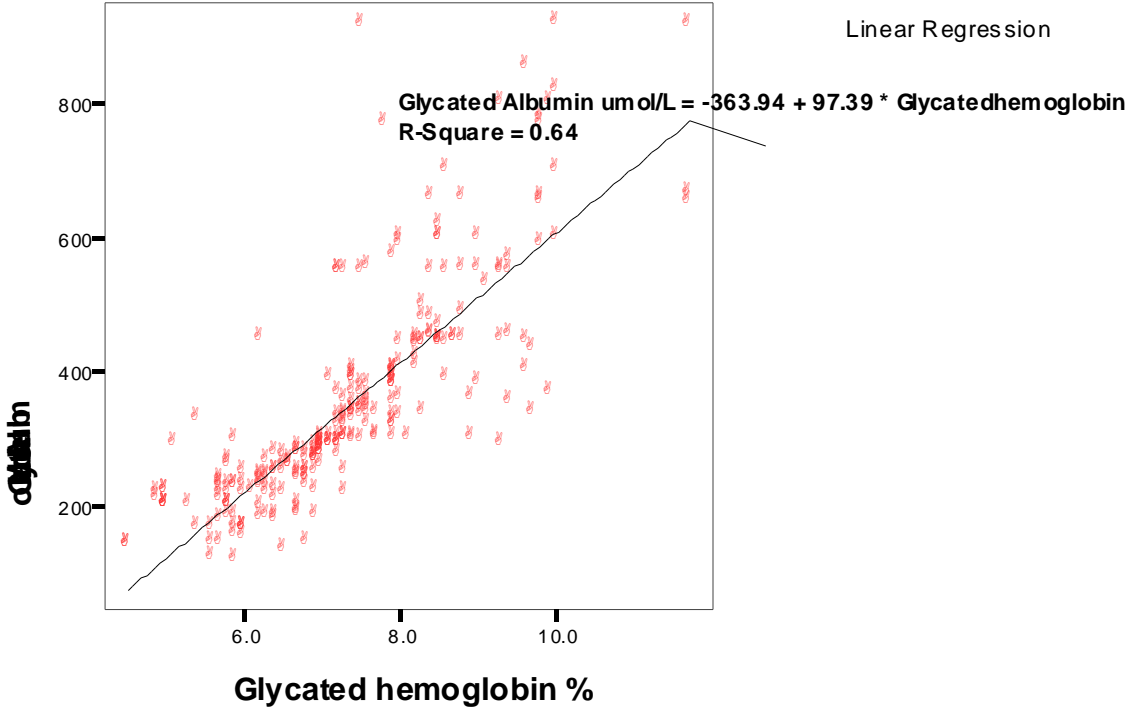
The linearity of the correlation is represented in the equation. The  $R^2$  value is 0.01. There is no correlation between RBS and HbA1c (Figure 10).

Figure 11: Correlation between Random Blood Sugar and Glycated Albumin



The linearity of the correlation is represented in the equation,  $RBS=9.07+0.00(GA)$ . The  $R^2$  value is 0.00. There is no correlation between blood sugar and Glycated Albumin (figure 11).

Figure 12: Correlation between Glycated Albumin and Glycated hemoglobin



The linearity is represented in the equation Glycated Albumin=3.63+97.39 (HbA1c). The R<sup>2</sup> value is 0.64. There is a positive correlation between Glycated Albumin and HbA1c (figure12).



## **DISCUSSION**

The Study population consisted had a mean age of 52 yrs with a female preponderance of 60.4%, this is similar to a study done by Otieno et.al in the same clinic in 1998 which found mean age of 52.5yrs (14-92) and female population of 52.6% (7).

Majority of the patients had type 2 diabetes, 214 (82.3%), while only 46 (17.7%) had type 1, this corresponds to literature which shows that type 2 diabetes constitutes 85-90% as compared to type 1 which is 10-15% (1).

Age and body mass index was significantly lower in type 1 Diabetics. This is similar to a study done by Kazutomi et .al in their study entitled: *Glycated Albumin is a better indicator of glucose excursion than Glycated Hemoglobin in Type1 and Type 2 Diabetes, (2008)*. According to this study the mean age for type 1 was 27yrs with SD of 7.4 and the mean for body mass index was 22.2. According to literature, Type 1 diabetic patients are diagnosed at a younger age because the total or near total lack of insulin production makes patients to present early with features of hyperglycemia. Majority of the type 2 diabetics usually present later, after 40yrs of age and The lowest BMI in the study population was 16.5 while the highest was 42. The mean was 27.3 with a standard deviation of 5. This is similar to Mwenda et .al study( 2005) where the mean body mass index was 28.7 with SD of 6. The majority of the patients were in the overweight category (25-29yrs).

A large number of patients were on insulin, 100 (38.5%) followed by those on oral glucose lowering agents, mainly metformin. The patients on combined therapy were 55 (21.2%).This differed from a study by Mwenda et.al which showed that majority of the patients were on oral hypoglycemic(76 %) while those on insulin were 13% but this is because Mwenda et. al study was done among type 2 diabetics only while this study included both type 1 and type 2 diabetics.

The main method of monitoring diabetes mellitus in the Kenyatta clinic is use self monitoring of blood glucose. This is because the test is cheaper and within the reach of yje largely poor population. After analysis of the plasma glucose the results showed that, the population with good glyceimic control constituted 156 (60%).,(Figure 5). Blood glucose monitoring measures a point in time glucose concentration in blood. Self monitoring of blood sugar (SMBG) can only provide a snapshot of blood glucose levels and does not monitor glycation, hence has minimal benefit in improving glyceimic control. The Fremantle study of 1,286 type 2 diabetes patients of over 5 years as well as a study of nearly 3,000 type 2 patients on oral medication or diet alone in Germany and Austria found no benefit for daily blood glucose testing regardless of treatment (8).

Another study done by Farmer in 2009 found little benefit for SMBG. In this study, type 2 patients with non-insulin treated diabetes were divided into three groups and followed for 12 months. All were given the same education as to how they could maintain or improve their condition: diet, exercise, etc. One group was given education and HbA1c testing every 3 months. The second group was given in addition a blood glucose meter, trained on its use, and told to test themselves 2 days a week and call a doctor if their results were above or below certain values. The third group was further given extensive training in using and interpreting the meter and encouraged to use it for multiple daily tests and to try to coordinate their lifestyle choices with meter results. After 12 months, the study found no significant improvement in glyceimic control, for any group, in spite of setting conditions for the intensive group into a framework that, based on psychological theory, should have optimized its effect

Self monitoring of blood glucose (SMBG) is especially important for patients treated with insulin to and prevents asymptomatic hypoglycemia and hyperglycemia. For most patients with type 1 diabetes and pregnant women taking insulin, SMBG is recommended three or more times daily (ADA) several recent trials have called into question the clinical utility and cost-effectiveness of routine SMBG in non–insulin-treated patients.

In addition to self monitoring of blood glucose some of the patients use HbA1c to monitor their glycaemic control after every 3 months. Results from this study showed that majority of the patients had good glycaemic control 170 (65.4%) (Figure 6). Only 90 (44.6%) had poor glycaemic control. This is different from study done by Otieno et.al which was assessing the quality of glycaemic control on ambulatory diabetic patients attending the clinic at Kenyatta National Hospital and found out that more than 60% of the patients attending the clinic had poor glycaemic control. They also found out that the group with poorest level of glycaemic control was that on oral glucose lowering agents while those with the best control were those on diet only possibly because of fair endogenous insulin production. Poor glycaemic control was presumed to be due to sub-optimal medication and deteriorating diabetes. (7)

HbA1c measurements have represented the gold standard for the evaluation of glycaemic control in diabetic patient for the past 30 years. DCCT demonstrated that each 1% increase in HbA1c is associated with an increase in mean blood glucose concentrations of 2mmol/l and this increased the risk of progression and development of micro-vascular complications in DM (10)

The expert panel of National committee for Quality Assurance (NCQA) has recently developed new targets of HbA1c target of <8% they considered that target of <6.5% is difficult to achieve. The scoring was thereby revised for the updated standard so that the percentage of patients with acceptable levels HbA1c <8% are 40%. It is recommended that there be need to establish population specific cut-off thresholds according to ethnicity, age, gender and prevalence of DM.

The ADA recommends assessing HbA1c at least 2 times a year in patients who are meeting treatment goals and have stable glycaemic control and quarterly in those patients whose hypoglycaemic therapy has changed or those who are not meeting glycaemic control goals.

From the findings, there was no correlation between the RBS and HbA1c this differs from Nathan D et.al who found out that there is correlation between average plasma glucose and HbA1c with the formula  $(28.7 \times A1C - 46.7 = eAG)$  which is recommended by ADA. Likewise no correlation was found between RBS and Glycated Albumin. This could be because in the Nathan

study the plasma glucose was averaged after running serial glucose tests while in this study the random blood sugar were run only once.

The results according the glycaited albumin showed that majority of patients 157 (60.6%) had poor glycemic control while 102 (39.4%) had good glycemic control (Figure 7).

Glycated Albumin has been reported as a rapid and useful indicator of glycemic control since the turnover of serum albumin is much shorter (half life of 17 days) than that of HbA1c.

Circulating albumin is strongly glycated at 4 sites of lysine residues and the glycation reaction occurs ten times more than in HbA1c. (17) (18). This implies that glycaemic fluctuation and excursion would influence glycation in albumin strongly.

Another study which had differing results was a Japanese study which was finding out whether GA was a more useful tool to monitor rapidly changing blood glucose than HbA1c. The study was performed on patients hospitalized for diabetes control (51 men & 47 women). Patients were administered oral anti-diabetic drugs and 4-point SMBG tests daily then 7-point SMBG tests were done the third and tenth hospital day. GA & HbA1c were performed the second and thirteenth hospital day. Results from the second day demonstrated a good correlation of blood glucose with HbA1c & GA ( $p=0.0001$ ). However, on the thirteenth day only GA correlated well with blood glucose ( $p=0.0001$ ) as opposed to HbA1c ( $p=0.019$ ). The study concluded that GA measurement is more accurate for determining rapidly changing blood glucose than HbA1c.

More recently, published studies in Japan, using a laboratory-based methodology for measuring glycated albumin have confirmed the clinical utility of glycated albumin as methodology for diabetes monitoring. A study of 18 type 2 diabetic patients for 16 weeks as they progressed from untreated severe hyperglycemia ( $HbA1c \geq 9.0\%$ ) to good glycemic control ( $HbA1c \leq 6.5\%$ ) by intensive insulin treatment found that GA decreased more rapidly than HbA1c during intensive insulin therapy, but the percent reduction of HbA1c eventually corresponded with that of GA by 16 weeks after the start of treatment. This result

demonstrates that GA provides a more responsive indication of therapeutic treatment than the HbA1c test (26)

In a similar study by Barry I. et al looked at comparison of Glycated Albumin and HbA1c concentrations in diabetic subjects on peritoneal and hemodialysis. In this study, 470 diabetic patients on dialysis were recruited; out of these 212 were new patients. On the new patients, HbA1c and %GA were run, it was found out that HbA1c were falsely low in patients with end stage renal disease whether they were on hemodialysis or peritoneal dialysis. The GA assays seemed to offer improved accuracy and not affected by dialysis. (21)

Lastly, the results show that there is a positive correlation between GA and HbA1c. The linearity is represented in the equation Glycated Albumin=3.63+97.39 (HbA1c). The R<sup>2</sup> value is 0.64. This compares to Nathan et.al study which showed that correlation coefficient between HbA1c and GA was 0.747 and was highly significant ( p < 0.001). The results show that Plasma glucose correlated well with both HbA1c and GA, although correlation coefficients were not large, likely due to the large variation in plasma glucose after meals. The results also show that there was better correlation between Plasma glucose and GA compared to HbA1c.

In a study done by Yasuhiro et al, it was found out that HbA1c had better correlation with GA using the measurement error model (MEM) of linear regression analysis. This model deals with statistical analysis in cases where data for both x and y contain relatively large errors. The sources of these measurement errors include in HbA1c and GA derive from the fact that neither of the values accurately represent the mean plasma glucose level due to various factors affecting their plasma levels. There are assay errors, errors arising from differences in the delay time of HbA1c and GA after plasma glucose change, errors arising from inter-individual distribution of the lifespan of RBCs and half life of serum albumin and complications affecting them. (25)

## **CONCLUSIONS AND RECOMMENDATIONS**

### ***CONCLUSIONS***

1. Random blood sugar analysis showed that, the population with good glycemic control constituted 156 (60%). In addition, majority of the patients had good glycemic control 170 (65.4%) based on the HbA1c assay
2. In this study there was no correlation between Random blood sugar with either HbA1c or Glycated Albumin.
3. There was correlation between HbA1c and Glycated Albumin with  $R^2$  value of 0.64.

### ***RECOMMENDATIONS***

The study found good correlation between HbA1c and Glycated Albumin which would support its utilization in monitoring glycemic control, however there is need for further studies to be done on characteristics of glycated albumin test and reference range validation in order to consider introducing it as a method of monitoring medium term glycemic control.

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### **Appendix I: Introduction and objectives of the study:**

I am Dr. Lotodo, a Masters student at the Department of Human Pathology at the University of Nairobi and conducting a study on Diabetes Mellitus. This is a chronic disease that leads to persistently high blood sugar levels. Poor glycemic control may lead to complications of diabetes in the kidneys, heart, retina and foot among others. The study aims to:

- i. Compare use of two blood components (glycated hemoglobin and glycated albumin) in diabetic control.

#### Benefits and risks of the study to you:

By participating in it, you will benefit by:

- o Having examinations and laboratory tests done on you at no added cost.
- o A report on your glycemic control being sent to your physician
- o Receiving appropriate advice and intervention measures undertaken to stop/ reverse progression to Diabetes complications.

Risk: Blood (2mls) will be drawn from the antecubital vein. The needle prick may be painful.

If you consent to participate, you will:

- Sign a consent form/assent (Appendix I), and answer some questions contained in the screening and study questionnaire.

Participation in this study is voluntary and you can withdraw at any time. Any information given to us will remain confidential. You may ask me any questions regarding this study now and at any time during the study. In case you have questions relating to the study, kindly contact.

1. Dr. Lotodo T.L.C                                  0722550807 (PI)
2. The Secretary to the Ethical Committee KNH Tel. Nos. 276300 Ext. 44102

**Appendix II: CONSENT FORM**

**COMPARATIVE STUDY OF GLYCATED ALBUMIN AND GLYCATED HEMOGLOBIN LEVELS AS A  
MEASURE OF CONTROL IN DIABETIC PATIENTS ATTENDING OUTPATIENT CLINIC AT  
KENYATTA NATIONAL HOSPITAL**

I ..... after reading and /or being explained to on the  
study purpose by Dr. Lotodo T.L.C, hereby give informed consent to participate in the study

I am aware that I can withdraw from this study without losing the health care benefits to  
which am entitled to at KNH.

Signature/thumb print .....Date.....

**Appendix III: Study Questionnaire**

**Comparative study of Glycated Albumin and Glycated Hemoglobin levels as a measure of control in Diabetic patients attending outpatient clinic at Kenyatta National Hospital**

Date ...../...../.....

Study number

Hospital number

Social Demographic data

1.Name\_\_\_\_\_

2.Age (yrs)

3.Gender

(1)M

(2) F

4.Medical history

a).Type of DM

(1).Type 1

(2).Type 2

b).Duration since diagnosis

(1). 0-5years

(2). 6-10yrs

(3). >10yrs

c).Mode of Treatment

- (1). Insulin
- (2). Oral glucose lowering agents
- (3).Combined (insulin and OGLA's)
- (4).Alternative therapy
- (5). Diet only

5.Physical Examination

- (1).Height(cm)
- (2).Weight(Kg)
- (3).BMI

6.Laboratory

- (1).Fasting blood sugar (mmol/l)
- (2). Hb(g/dl)
- (3).HbA1c (%)
- (4).GA (%)

7.Glycemic control

- (1).Good
- (2).Poor

# Appendix IV: Methodology for Glycated Hemoglobin

## GLYCOHEMOGLOBIN HbA1-TEST Fast Ion-Exchange Resin Separation Method

Package Sizes			
[REF]	10657	20 Tests	Complete Test Kit
	10658	100 Tests	Complete Test Kit
	10259	2 x for 1 ml	Glycohemoglobin Controls normal and abnormal

### IVD

### Method

Glycohemoglobin is irreversibly and progressively formed in the erythrocytes throughout the normal cells 120-day life span. Since the concentration of glycohemoglobin in the erythrocytes reflects the average blood glucose level of the past 4 to 6 weeks and is stable for the life of the erythrocytes, the measurement of glycohemoglobin provides a very valuable test for assessing the long term control of diabetic patients.

### Reaction Principle

Whole blood is mixed with a lysing reagent containing a detergent and borate ions. Elimination of the labile Schiff's base is thus achieved during the hemolysis. The hemolysate is then mixed for 5 minutes with a weakly binding cation exchange resin. During this time, HbA0 binds to the resin. A special resin separator is used to remove the resin from the supernatant fluid which contains the HbA1. The glycohemoglobin percentage of total hemoglobin is determined by measuring the absorbance of the glycohemoglobin and of the total hemoglobin fraction at 415 nm or Hg 415 nm in comparison with a standard glycohemoglobin preparation carried through the test procedure.

### Contents

[REF]	10657	10658	
[LYSE]	10 ml	5 x 10 ml	
[RGT]	20 x 2.5 ml	100 x 2.5 ml	
[STD]	1 x for 1 ml	1 x for 1 ml	
[CUP]	20	100	
[SEP]	20	100	
[LYSE]	Lysing reagent (pH 7 ± 0.1)		
	Borate		1 mol/l
	Detergents		0.25 %
[RGT]	Ion exchange resin (prefilled in plastic tubes) Imidazole buffer (pH 7.6 ± 0.1) 30 mmol/l Borate		150 mmol/l
[STD]	for 1.0 ml Standard (freeze dried hemoglobin) human, concentration see label		
[CUP]	Plastic tube for hemolysis		
[SEP]	Resin separators		
[REF]	10259		
[GCN]	for 1.0 ml Glycohemoglobin control (normal) human, concentration see label		
[GCA]	for 1.0 ml Glycohemoglobin control (abnormal) human, concentration see label		

### Reagent Preparation and Stability

[RGT]: Ready to use. Store at 2...25°C.  
[LYSE]: Ready to use. Stable for 2 months after first opening when stored at 2...25°C. Mix well before use. Pipette 0.5 ml each into individually labelled [CUP] for sample, [STD], and controls.  
[STD], [GCN] and [GCA]: Store at 2...8°C. Reconstitute with 1.0 ml of distilled water. Allow to stand for 30 min. with occasional mixing. Use fresh or stored frozen in aliquots. The reconstituted reagents are stable for 30 days when stored frozen at -20°C or below. Thaw only once. Mix well prior to use. Handle exactly like specimen.

### Specimen

Use whole blood with EDTA as anticoagulant. Specimens are stable for 1 week at 2...8°C. Mix well before use.

### Assay

Wavelength: 415 nm or Hg405  
Temperature: 15...25°C  
Measurement: against water

### Procedure

<b>Step 1 Hemolysis</b>
Pipette into prefilled [CUP] 100 µl [STD], sample, [GCN] or [GCA]
Mix. Incubate 5 min at 15...25°C (Note 2)
<b>Step 2 HbA1 Determination (Note 3)</b>
Pipette into labelled [RGT] 100 µl hemolysate from Step 1
Insert [SEP] so that the rubber sleeve is approx. 1cm above the surface of the resin suspension. Mix on a haematology mixer for 5 min. Push [SEP] down until the resin is firmly packed. Pour supernatant into cuvette.
Read absorbance A <sub>HbA1 [STD] /sample/control</sub>
<b>Step 3 Total Hemoglobin Determination</b>
Pipette into labelled tubes 20 µl hemolysate from Step 1
Add 5 ml of distilled water
Mix carefully
Read absorbance A <sub>total Hb [STD] /sample/control</sub>

### Calculation of the HbA1 Content

#### Factor F Determination by Use of [STD]:

The glycohemoglobin percentage (% HbA1 [STD]) is stated on the label under %.

$$F = \frac{A_{\text{total Hb [STD]} \times \% \text{ HbA1 [STD]}}{A_{\text{HbA1 [STD]}}$$

#### Glycohemoglobin Content of the Sample:

$$\% \text{ HbA1 sample} = F \times \frac{A_{\text{HbA1 sample}}}{A_{\text{total Hb sample}}}$$

### Clinical Interpretation

Patients	% HbA1
Well controlled metabolism or stabilised diabetics	4.5 – 7.0
Diabetics, insufficiently controlled or with metabolic imbalance	≥ 8.5

### Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via:  
[www.human.de/data/gb/vr/su-glych.pdf](http://www.human.de/data/gb/vr/su-glych.pdf) or  
[www.human-de.com/data/gb/vr/su-glych.pdf](http://www.human-de.com/data/gb/vr/su-glych.pdf)

### Notes

- Results are not influenced by temperature variations. Run [STD] at suitable intervals, at least once per kit.
- Diabetics with metabolic imbalance may have extremely high levels of the labile aldimine form. For total elimination of this in-stable fraction increase the incubation time in Step 1 to 15 minutes.
- Mix [RGT] well before use by swirling to ensure reproducibility of the test.
- With no haematology mixer available, [RGT] may be swirled manually or by using a vortex mixer. Shake [RGT] several times for 10-15 seconds during Step 2.
- Final diagnosis should not be made on the result of a single test, but should be based on a correlation of test results with other clinical findings.
- [STD], [GCN] and [GCA] have been tested and found to be negative for HBsAg, HCV and HIV antibody. They should however be handled carefully as potentially infectious material.
- [LYSE] contains sodium azide (10 mmol/l). [RGT] contains thimerosal (0.1 g/l). Do not swallow. Avoid contact with skin and mucous membranes.

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SU-GLYCH INF 1065701 GB 02-2009-15

CE  
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# Appendix V: Methodology for Glycated Albumin



Diazyme Laboratories  
 12889 Gregg Court  
 Poway, CA 92064, USA  
 Tel: 858-455-4768 / Fax: 858-455-3701  
 Email: support@diazyme.com  
 Website: www.diazyme.com

## Glycated Serum Protein Assay (GSP; Glycated Albumin)

### Configuration

The Diazyme Glycated Serum Protein (GSP; Glycated Albumin) reagent (GlycoGap™) is provided in bulk and the following kit configuration:

Configuration	Catalog No.	Kit Size	Number of Test
Universal	DZ112B-K	R1: 2 x 23 mL R2: 2 x 7.5 mL	200

### Intended Use

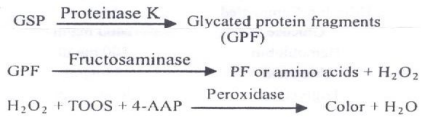
Diazyme Glycated Serum Protein Assay in conjunction with Diazyme Glycated Serum Protein single calibrator, are intended for the quantitative determination of glycated serum proteins (GSP; glycated albumin; fructosamine) in serum. The measurement of glycated serum proteins is useful for monitoring diabetic patients. For *in vitro* diagnostic use only.

### Clinical Significance<sup>1</sup>

Fructosamine is formed due to a non-enzymatic Maillard reaction between glucose and amino acid residues of proteins. In diabetic patients, elevated blood glucose levels correlate with increased fructosamine formation. Glycated serum proteins (GSP; glycated albumin; fructosamine) are a medium term indicator of diabetic control (2-3 weeks).

### Assay Principle<sup>2</sup>

The Diazyme Glycated Serum Protein Assay uses proteinase K to digest GSP into low molecular weight glycated protein fragments (GPF), and uses Diazyme's specific fructosaminase™, a microorganism originated amadoriase to catalyze the oxidative degradation of Amadori product GPF to yield PF or amino acids, glucosone and H<sub>2</sub>O<sub>2</sub>. The H<sub>2</sub>O<sub>2</sub> released is measured by a colorimetric Trinder end-point reaction. The absorbance at 600 nm is proportional to the concentration of glycated serum proteins.



### Materials Required But Not Provided

- GSP calibrator set (DZ112B-CAL) includes saline with preservative (DZ112B-S0V) for use as calibrator 0 and lyophilized calibrator 1 (DZ112B-S1V).
- Diazyme bi-level GSP controls (DZ112B-CON) are recommended.

### Reagent Composition

- R1: Enzyme/substrate reagent containing Good's buffer, 4-AA, Enzymes and stabilizers
- R2: Enzyme/substrate reagent containing Good's buffer, enzymes, TOOS, HRP, Geneticin, and stabilizers

### Reagent Preparation

Reagent 1 and Reagent 2 are ready to use. Reagents from different lots should not be interchanged.

### Reagent Stability and Storage

The reagents are stable at 2-8°C until the expiration date noted on the labels. The reagents are stable for 4 weeks once opened and stored on-board or refrigerated at 2-8°C.

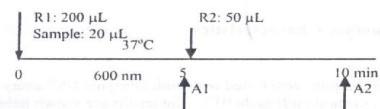
### Specimen Collection and Handling

Use fresh patient serum samples. Serum should be separated from cells immediately after collection. Samples can be stored at 2-8°C for 2 weeks or at -20°C for up to 4 weeks.

### Precautions

- Specimens and reagents containing human sourced materials should be handled as if potentially infectious, using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395).
- As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
- Avoid swallowing and contact with skin or mucous membranes.
- Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product. To obtain an MSDS, please contact our customer service department at 858-455-4768.

### Assay Procedure



Application sheets for use of Diazyme Glycated Serum Protein Assay on automated clinical chemistry analyzers are available upon request. Please call 858-455-4768 or email: support@diazyme.com.

### Calibration

The Diazyme Glycated Serum Protein Assay requires weekly calibration. Diazyme GSP calibrator set (DZ112B-CAL) includes saline with preservative (DZ112B-S0V) for use as calibrator 0 and lyophilized calibrator 1 (DZ112B-S1V). Enter 0  $\mu\text{mol/L}$  for calibrator 0 and calibrator lot specific value provided on the certificate of analysis sheet for calibrator 1 on analyzer to perform calibration. Diazyme GSP calibrator is intended for use with Diazyme Glycated Serum Protein Assay reagents (DZ112B). The calibrator 1 is in lyophilized form and stable at 2-8°C until the expiration date noted on the label. Reconstitute lyophilized contents of calibrator 1 with 1ml of distilled water per instruction on certificate of analysis. To ensure complete reconstitution, equilibrate vial at room temperature for 30 minutes with gentle swirling a few times before first use, make sure all the contents are dissolved. Reconstituted calibrator 1 is stable for 14 days when stored at 2-8°C capped tightly.

### Quality Control

Diazyme bi-level GSP controls (DZ112B-CON) are recommended to use as daily quality control and can be purchased separately from Diazyme Laboratories. Users should follow the appropriate federal, state and local guidelines concerning the running of external quality controls and handling of bio-hazardous material. Diazyme bi-level GSP controls are in lyophilized form and stable at 2-8°C until the expiration date noted on the label. Reconstitute lyophilized contents of each vial with 1ml of distilled water per instruction on certificate of analysis. To ensure complete reconstitution, equilibrate vial at room temperature for 30 minutes with gentle swirling a few times before first use, make sure all the contents are dissolved. Reconstituted controls are stable for 14 days when stored at 2-8°C capped tightly.

### Results

Glycated Serum Protein Assay results are reported in  $\mu\text{mol/L}$ .

### Reference Range

Adults (20-60 years) have a reported normal range of 100-285  $\mu\text{mol/L}$ . It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

### Limitations

Roche fructosamine calibrators and controls may not be compatible with Diazyme Glycated Serum Protein Assay.

### Performance Characteristics

#### Accuracy

65 serum samples were tested with both Diazyme GSP assay and predicate method on Hitachi 917. The results are shown below:

	Diazyme vs. predicate on Hitachi 917
N	65
$r^2$	0.9966
slope	0.9542
Y intercept	14.57

#### Precision

The precision of the Diazyme GSP Assay was evaluated according to CLSI EP5-A guideline. In the study, two controls and two levels

of serum specimens containing about 200, 750, 250 and 375  $\mu\text{mol/L}$  fructosamine, respectively, were tested 2 runs per day in duplicates over 20 working days.

#### Within run precision

Samples	Control Level 1	Control Level 2	Serum Level 1	Serum Level 2
N	80	80	80	80
Mean ( $\mu\text{mol/L}$ )	204	751	251	373
SD ( $\mu\text{mol/L}$ )	2.2	4.9	1.9	2.4
CV (%)	1.1%	0.7%	0.8%	0.6%

#### With-laboratory Precision

Samples	Control Level 1	Control Level 2	Serum Level 1	Serum Level 2
N	80	80	80	80
Mean ( $\mu\text{mol/L}$ )	204	751	251	373
SD ( $\mu\text{mol/L}$ )	2.4	5.6	3.2	3.7
CV (%)	1.2%	0.7%	1.3%	1.0%

### Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ of Diazyme GSP Assay were determined according to CLSI EP17-A. The LOD was determined to be 7.2  $\mu\text{mol/L}$  and LOQ was 13.0  $\mu\text{mol/L}$ .

### Linearity

Nine levels of linearity set were prepared by diluting a sample containing 1579  $\mu\text{mol/L}$  Fructosamine with saline according to CLSI EP6-A. The linearity set prepared was analyzed on Hitachi 917 over up to 1354  $\mu\text{mol/L}$ . allowable systematic error (Sea) was 3.5%.

Analytical measurement range of Diazyme Glycated Serum Protein Assay is 21.0 - 1354.0  $\mu\text{mol/L}$ .

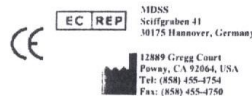
### Interferences

The following interfering substances produce less than 10% deviation when tested at the indicated concentrations.

Ascorbic Acid	5 mg/dl
Bilirubin	7.5 mg/dl
Bilirubin Conjugated	5 mg/dl
Glucose	2400 mg/dl
Hemoglobin	200 mg/dl
Uric Acid	35mg/dl
Triglyceride	2000 mg/dl

### References

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## Appendix VI: Approval letter KNH/UoN Ethics and Research Committee



Ref: KNH-ERC/ A/98

Dr. Lotodo T.L.C.  
Dept. of Human Pathology  
School of Medicine  
University of Nairobi

Dear Dr. Lotodo

**RESEARCH PROPOSAL: "COMPARATIVE STUDY OF GLYCATED ALBUMIN AND GLYCATED HEMOGLOBIN LEVELS AS A MEASURE OF CONTROL IN DIABETIC PATIENTS ATTENDING OUT-PATIENT CLINIC AT KENYATTA NATIONAL HOSPITAL" (P24/1/2011)**

This is to inform you that the KNH/UON-Ethics & Research Committee has reviewed and **approved** your above revised research proposal for the period 21<sup>st</sup> April 2011 – 19<sup>th</sup> April 2012.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimens must also be obtained from KNH/UON-Ethics & Research Committee for each batch.

On behalf of the Committee, I wish you a fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of the data base that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely,

**PROF A N GUANTAI**  
**SECRETARY, KNH/UON-ERC**

c.c. The Deputy Director CS, KNH  
The HOD, Records, KNH  
Supervisors: Wandolo George O., Dept of Human Pathology, UON  
Prof. Christine Kigundu, Dept. of Human Pathology, UON  
Prof. C.F.Otieno, Dept. of Medicine  
Dr. P. Ngugi, Consultant Physician, KNH

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21<sup>st</sup> April 2011