FASTING BLOOD GLUCOSE AND GLYCOXYLATED HAEMOGLOBIN LEVELS IN RANDOMLY SELECTED GHANAIAN DIABETIC PATIENTS – THE CLINICAL IMPLICATIONS


1Department of Biochemistry, Kwame Nkrumah University of Science and Technology.
2Department of Molecular Medicine, School of Medical Sciences, Kwame Nkrumah University of Science and Technology.
3Department of Medicine, Komfo Anokye Teaching Hospital

ABSTRACT
This work involved the measurement of fasting blood glucose (FBG) and glycosylated haemoglobin (HbA1c) levels of diabetes mellitus patients as an index of glycaemic control. It was a prospective case-finding study using laboratory and general practice records. The subjects were confirmed diabetic patients, attending a Diabetic Clinic at the Komfo Anokye Teaching Hospital, Kumasi, Ghana. The fasting blood glucose levels were measured in millimolar concentration and corrected glycosylated haemoglobin (HbA1c) levels expressed as percentages. The mean level of fasting blood glucose (± standard deviation) for the non-diabetics was 4.91 ± 1.08 mmol/L and the corresponding mean value for the HbA1c was 5.40 ± 0.84%. There was a linear correlation between the fasting blood glucose and HbA1c. Out of the 99 diabetics, 9 of them had near-normal levels of HbA1c, while 64 had mean values between 12 and 16%. There was generally high levels of glycosylated haemoglobin in the majority of patients studied, reflecting their poor glycaemic control. This suggests a relatively large proportion of the diabetics could be predisposed to microvascular complications, while a small group with near-normal HbA1c levels could be prone to hypoglycaemic complications.

Keywords: Diabetes mellitus, hyperglycaemia, glycosylated haemoglobin, microvascular complications, glycaemic control.

INTRODUCTION
The measurement of glycosylated haemoglobin (HbA1c) provides an objective and quantitative index of diabetic control (Ashby et al., 1985; Peterson et al., 1995). Glycosylated haemoglobin reflects glycaemic control in diabetic patients over the preceding 1-2 months (Holman et al., 1987). Monitoring the degree of glycosylation may have particular relevance, as glycosylation of many proteins, e.g. anti-thrombin III and fibrin alters their normal function (Brownlee et al., 1984; Cohen and Ku 1980), and may contribute to the pathological processes leading to diabetic complications, like microvascular disorders (UK Prospective Diabetes Study Group 33). Even though the achievement and maintenance
of blood glucose concentrations as near normal as possible are major targets of modern diabetic care (UK Prospective Diabetes Study Group, 1988), this increases the frequency of hypoglycaemia (Amiel 1998). Hypoglycaemia, the most common acute complication of type 1 diabetes, usually develops rapidly, with its effects ranging from mild symptoms to brain damage or death (Allen et al., 2001). Cryer et al., (2003) reports that the rates of severe hypoglycaemia in type 2 diabetes are substantially lower than those in type 1, but the consequences of the hypoglycaemia in type 2 is more serious as older people tend to have more morbidities.

HbA1c is one of the four minor haemoglobins, which can be identified by cation exchange chromatography (Mayer and Freedman, 1983). Its use as a diagnostic tool for diabetes mellitus was proposed by Gonen and Rubinstein (1978).

HbA1c is formed throughout the life of the erythrocyte by a post-translational modification of the haemoglobin molecule. The chemistry of the non-enzymatic glycosylation has been extensively studied (Holmquist and Schroeder, 1966). Glucose is linked to the N-terminal valine residue of the β-chain by a two-stage reaction. Initially, the aldehyde group of glucose participates in the formation of a Schiff base linkage to form a labile aldimine. Most of this subsequently dissociates to give HbA0 and free glucose, while a small proportion of the aldimine undergoes an irreversible Amadori rearrangement to form a stable ketoamine, HbA1c (Bunn et al., 1975; Ashby et al., 1985). Other minor adducts of haemoglobin are HbA1a and HbA1b, which are thought to be formed by the attachment of sugar phosphates to the N-terminus of β-chain, whereas HbA1b appears to be a deamination product of α-glutamine and α-sparagine (Ashby et al., 1985). The total concentration of these minor components is about ten fold lower than HbA1c and does not appear to be elevated in diabetics (Mayer and Freedman, 1983).

Despite the usefulness of combining the two parameters of FBG and HbA1c as diabetic control indices, the measurement of HbA1c is generally uncommon in developing countries like Ghana. The current advances in health care delivery among diabetics make it imperative that a more definitive means of diagnosis and prognosis should be adopted, hence the use of the measurement of HbA1c in this study. Furthermore, knowing the normal range of glycosylated haemoglobins, and the cut-off values for some clinical conditions, we would be in a position to predict the degree of glycaemic control of diabetics in relation to compliance to drug therapy or change in lifestyle, or both. In addition, the extent of glycaemic control could be matched with the actual clinical picture of the diabetic patients vis-à-vis the incidence of hypoglycaemic and microvascular complications.

In the present study, measurements of HbA1c of randomly selected patients with long-standing diabetes mellitus were made using affinity chromatography. Also measured were the levels of fasting blood glucose. Concurrently, the FBG and HbA1c of non-diabetic controls were measured.

**MATERIALS AND METHODS**

**Patients**

The study involved ninety-nine (99) diabetic (mostly type II or non-insulin dependent diabetes mellitus) and twenty-six (26) non-diabetic volunteers of both sexes. The diabetics were out-patients of the Diabetes Clinic at the Komfo Anokye Teaching Hospital (Kumasi, Ghana), who had been referred to the Clinical Biochemistry Laboratory of the hospital. The controls were hospital workers and students. The enrolment of both the diabetics and volunteers was done randomly with their informed consent.

Blood samples were taken after an overnight fast to measure the fasting blood glucose and glycosylated haemoglobin. The blood samples were drawn from the antecubital vein into sample
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tubes containing ethylenediaminetetraacetic acid (EDTA), an anti-coagulant. The FBG was determined using the glucose oxidase method. Prior to the assay of HbA1c, the EDTA-anticoagulated blood was stored at 4°C for three days. A pre-packed affinity chromatography minicolumns from Sigma Diagnostic (St. Louis, MO, USA) was used for the assay of HbA1c. All measurements were made in duplicate.

STATISTICAL ANALYSIS

The means and standard deviations of the fasting blood glucose and glycosylated haemoglobin were calculated for significance using Student’s t-test. In addition, by means of linear regression analysis the correlation coefficients between the FBG and HbA1c in the normal and diabetic patients were determined.

RESULTS

Figure 1 shows the linear relationship (y = 0.64x + 5.0) between the fasting blood glucose and glycosylated haemoglobin levels of the diabetics. Correlation analysis of the FBG and HbA1c showed a weak relation between the two parameters (r = 0.076; p < 0.01). The mean level of fasting blood glucose for the non-diabetics was 4.91 ± 1.08 mmol/L. The reference range for the fasting blood glucose in the non-diabetics was 2.75 - 7.07 mmol/L. The corresponding mean value for the glycosylated haemoglobin was 5.40 ± 0.84%. The range for the glycosylated haemoglobin was 3.72 - 7.08%. In the case of the diabetics, the fasting blood sugar ranged between 3.9 to 22.5 mmol/L, while the glycosylated haemoglobin was between 5.3 and 25.5%.

Figure 2 shows the relative distribution of HbA1c and mean fasting blood glucose in the diabetics. About 9% of the diabetics had their levels of HbA1c within the reference range obtained in this study. On the other hand, 64.6% of the patients who had HbA1c levels from 12 to values above 16% had their mean fasting blood glucose between 11.45 and 16.65 mmol/L.

DISCUSSION

The highest level of fasting blood glucose (FBG) in the non-diabetics or normal subjects was 6.3 mmol/L while the lowest was 3.1 mmol/L. On the other hand, in the diabetics, the highest FBG was as high as 22.5 mmol/L while the lowest was 3.9 mmol/L. The mean HbA1c level for the controls in this study, 5.40% is comparable to a mean of 4.73% recorded for 71 healthy non-diabetic Nigerian HbAA subjects (Reid et al., 1992).

It has been suggested (Baynes et al., 1984) that each laboratory should determine its own reference range of HbA1c, even when using the same method (Reid et al., 1992). One way of assessing the precision of a method is to determine the within-run or between-run coefficient of variation. A coefficient of variation of less than 5% denotes high precision.

From Figure 1 there is a linear correlation between the fasting blood glucose and the glycosylated haemoglobin for the diabetics (HbA1c = 0.64FBG + 5.0). That there is a scatter in the plot suggests that not all the increases in glucose levels would lead to a corresponding increase in HbA1c and such random glucose assays may provide false and inaccurate representation of the patients’ glucose levels, particularly so when it is a single measurement as was done in this study.

Other factors that caused deviation from linearity may include the type, timing and frequency of dietary intake (Otiendo et al., 2002). Another possibility is the manipulations of patients (Ashby et al., 1985) who attempt to deceive their physicians by taking prescribed drugs close to the days they have appointments with the physicians. From the distribution of the mean fasting blood glucose and HbA1c levels (Fig 2) it can be suggested that two types of complications are possible. For example, diabetic patients with normal or near-normal HbA1c levels are at much increased risk of experiencing serious symptomatic hypoglycaemia (Goldstein et al., 1982).
This group, constituting about 9% of the diabetic patients had a mean blood glucose level of 4.83 mmol/L and HbA1c levels less than 6%, might have suffered episodes of hypoglycaemia. On the other hand, 64.6% of the patients who had their HbA1c levels clearly above the normal range (from 12% to above 16%) could be prone to microvascular complications.

The main deduction which could be made from this work is that, based on the measurement of fasting blood glucose and HbA1c levels, 64.6% of the diabetic patients studied had poor glycaemic control. This is comparable to 60.5% obtained by Otieno et al., (2002) in a similar study in Kenya. It is worth noting that the highest levels of FBG and HbA1c (22.5mmol/L and 25.5%) were found in this group. The level of HbA1c depends on both the degree of hyperglycaemia and the duration (Gabbay et al., 1977; Bunn et al., 1978).

It has been suggested by Ashby et al., (1985) that poor glycaemic control could probably be due to their irregular attendance to the Diabetic Clinic, coupled with their non-compliance with drug therapy, dietary restriction or change in lifestyle. It was further pointed out that the measurement of HbA1c may provide a useful adjunct to the management of diabetic patients. A higher than anticipated level can help identify patients with sub-optimal control, thus providing objective evidence of treatment failure with oral hypoglycaemic agents.

Diabetic patients with high levels of glycosylated haemoglobin are prone to microvascular complications like nephropathy and retinopathy. It is those with poor diabetic control who usually suffer from these complications. A direct correlation between the level of HbA1c and the occurrence of complications has been reported elsewhere (UK Prospective Diabetes Study Group 33).

CONCLUSION
Using the dual diagnostic criteria of FBG and HbA1c, it has been shown that 64.6% of 99 patients in our study had poorly controlled diabetes mellitus, predisposing them to some microvascular complications. A smaller group, making up about 9% of the patients could be prone to hypoglycaemic complications.

Even though the number of subjects used in this study was small, the results nonetheless, compare favourably to those obtained in some African countries. Thus by using simple cost-effective procedure, it has been shown that a combination of FBG and HbA1c measurements provide a fair evaluation of patients glycaemic control. We therefore conclude that, for a proper assessment of diabetic status, measurements of glycosylated haemoglobins should be done, where possible.

REFERENCES
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