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

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ABSTRACT

This work involved the measurement of fasting blood glucose (FBG) and glycosylated haemoglobin (HbA_{1c}) 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 (HbA_{1c}) 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 HbA_{1c} was 5.40 ± 0.84%. There was a linear correlation between the fasting blood glucose and HbA_{1c}. Out of the 99 diabetics, 9 of them had near-normal levels of HbA_{1c} 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 HbA_{1c} levels could be prone to hypoglycaemic complications.

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

INTRODUCTION

The measurement of glycosylated haemoglobin (HbA_{1c}) 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.

HbA_{1c} 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).

HbA_{1c} 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 HbA_0 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 HbA_{1a1} and HbA_{1a2} which are thought to be formed by the attachment of sugar phosphates to the N-terminus of β-chain, whereas HbA_{1b} appears to be a deamination product of glutamine and asparagine (Ashby *et al.*, 1985). The total concentration of these minor components is about ten fold lower than HbA_{1c} and does not appear to be elevated in diabetics (Mayer and Freedman, 1983).

Despite the usefulness of combining the two parameters of FBG and HbA_{1c} as diabetic control indices, the measurement of HbA_{1c} 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 HbA_{1c} 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 HbA_{1c} 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 HbA_{1c} 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

tubes containing ethylenediaminetetraacetic acid (EDTA), an anti-coagulant. The FBG was determined using the glucose oxidase method. Prior to the assay of HbA_{1c}, the EDTA- anticoalgulated 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 HbA_{1c}. 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 HbA_{1c} 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 HbA_{1c} 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 HbA_{1c} and mean fasting blood glucose in the diabetics. About 9% of the diabetics had their levels of HbA_{1c} within the reference range obtained in this study. On the other hand, 64.6% of the patients who had HbA_{1c} 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 HbA_{1c} 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 HbA_{1c}, 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 that 5% denotes high precision.

From Figure 1 there is a linear correlation between the fasting blood glucose and the glycosylated haemoglobin for the diabetics ($HbA_{1c} = 0.64FBG + 5.0$). That there is a scatter in the plot suggests that not all the increases in glucose levels would lead to a corresponding increase in HbA_{1c} 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 (Otieno *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 HbA_{1c} levels (Fig 2) it can be suggested that two types of complications are possible. For example, diabetic patients with normal or near-normal HbA_{1c} 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 HbA_{1c} levels less than 6%, might have suffered episodes of hypoglycaemia. On the other hand, 64.6% of the patients who had their HbA_{1c} 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 HbA_{1c} 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 HbA_{1c} (22.5mmol/L and 25.5%) were found in this group. The level of HbA_{1c} 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 life style. It was further pointed out that the measurement of HbA_{1c}, 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 HbA_{1c} and the occurrence of complications has been reported elsewhere (UK Prospective Diabetes Study Group 33).

CONCLUSION

Using the dual diagnostic criteria of FBG and HbA_{1c} 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 HbA_{1c} 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.

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