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GLYCATED HAEMOGLOBIN AND GLYCAEMIC CONTROL OF DIABETICS IN ILORIN

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ABSTRACT
Objectives: With increasing adoption of Western Lifestyle in Nigeria, the incidence of Diabetes Mellitus is on the increase in the country with its attendant complications. The objective of this study was to determine the prevalence of patients at risk of developing diabetic complications in Ilorin, Nigeria, among our patients with diabetes mellitus.

Methods: A cross-sectional study of diabetic patients attending the University of Ilorin Teaching Hospital diabetic clinic was carried out. Glycated haemoglobin as an index of medium term glucose control was assayed in established diabetics. The result obtained was evaluated against the bench mark HbA_{1C} value of 7.2% for the development of complication.

Results: Fifty-six percent of the subjects were females and all of them were forty years and above in age. Only four percent of the patients were below the age of forty years. Seventy-two percent of the subjects had diabetes for less than 10 years. Only female patients had BMI values greater than 30kg/m^2. About 64% of the patients had HbA_{1C} value >7.2%. More males (73.7%) had HbA_{1C} 7.2% than females (64.5%) ($P<0.05$). The patients had a mean HbA_{1C} value of 8.0%, while the mean HbA_{1C} in the control was 5.2%. These two mean HbA_{1C} values gave a P-value of 0.0001 on Student t-test. The female diabetic patients had a mean HbA_{1C} value of 7.8% (SD=1.96) against the value of 5.1% (SD=1.13) for the female control patients (P-value of 0.0001). Similarly, the male patients and male control subjects had mean HbA_{1C} values of 8.1% (SD=1.96) and 5.6% (SD=1.00) respectively with P-value of 0.0001. The control subjects had a mean fasting blood glucose level of (± SD) 4.93 ± 1.09 mmol/L and the corresponding value for the diabetics was 8.5 ± 4.2 mmol/L. when these two values were compared we got a P-Value <0.05.

Conclusions: The mean HbA_{1C} values between the patients and the control subjects were significantly different. Diabetics in our environment with mean HbA_{1C} value of 8.0% are prone to developing complications because of poor glycaemic control. We therefore advise that, periodic estimation of glycated haemoglobin be carried out along side fasting blood glucose, in our diabetics.

Key Words: Glycated Haemoglobin, Type11 Diabetes, Glycaemic Control, Ilorin. *(Accepted 14 February 2008)*

INTRODUCTION
Glycation is the non-enzymatic addition of a sugar residue to amino groups of proteins. In the case of haemoglobin (Hb), this glycation can occur with the condensation of glucose to the N-terminal valine of the βeta-chain of HbA as in case of HbA_{1C}. The formation of glycated haemoglobin is irreversible, and its blood level depends on both the life span of the red blood cell and the blood glucose concentration[1].

Human adult Haemoglobin (Hb) usually consists of HbA (97% of total), HbA_2 (2.5%), and HbF (0.5%).

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Chromatographic analysis of HbA identifies several minor haemoglobins, namely HbA_{1a}, HbA_{1b}, and HbA_{1c}, which are collectively referred to as HbA_1, fast haemoglobins, glycohaemoglobins or glycated haemoglobins. HbA_{1C} is the major fraction of the fast haemoglobins, which accounts for the 3-6% of the total-haemoglobin[2].

Minor haemoglobin fractions representing glycation products are present in higher concentrations in diabetic patients than in non-diabetic patients [3]. Measurement of such glycated proteins as haemoglobin is a useful tool in monitoring long-term glucose control in individuals with diabetes mellitus[1]. The amount of HbA_{1C} is known to represent the integrated values for glucose over the preceding 6 to 8

weeks and provides therefore, an additional criterion for assessing glucose control[4]. This becomes very useful, knowing that the single most important risk factor for the development of diabetic-complication is sustained hyperglycaemia[5] . An additional advantage of glycated haemoglobin measurement is that, value is free of day-to-day glucose fluctuations and unaffected by exercise or recent food ingestion[1].

In estimating glycated haemoglobin level; either blood total HbA_1 or HbA_{1C} can be measured. Values for total HbA_1 and HbA_{1C} have been shown to have a high degree of correlation[1]. This is because HbA_{1c} is the major fraction, constituting approximately 80% of HbA_1.

Glycaemic control assessment in our institution for patient care is based solely on plasma glucose measurement. This is known to be limited by problems mentioned above. The aim of this work is to assess the status of glycaemic control among our patients with diabetes mellitus by determining their levels of glycated haemoglobin. This is with a view to gauging how well our patients are controlled, based on monitoring system that relies exclusively on fasting plasma glucose measurement.

PATIENTS AND METHOD

Five hundred patients who are established to have non-insulin dependent diabetes mellitus (NIDDM) and attending the diabetic clinic of University of Ilorin Teaching Hospital were recruited into this study by random selection. They were all on oral hypoglycaemic agents' glibenclamide and metformin. Those with treatment history less than six months were excluded. Those with a history suggestive of a haemolytic disorder (like sickle cell anaemia) or any known condition with shortened red blood cell survival were excluded.

The recruited patients had their age, sex, duration of illness, type of treatment for hyperglycaemia, weight (kg), height (metres) and body mass index (BMI) recorded. These patients were fasted overnight (between 10pm and 8 am) after which 6mls of blood was collected from each of them between 8.00am-9.00am. The collected blood was put in heparinized sample bottles and stored in the refridgerator at a temperature of 4^0C till assayed for glycated HbA_{1C} the following day.

To estimate the percentage HBA_{1C}, the ion-exchange-temperature independent chromatographic method, using microcolumns as developed by Biosystems Company of Spain was used[6,7].

Four hundred age and sex matched controls were randomly selected among the University of Ilorin staff members. Those found to be diabetic, or with a history suggestive of a haemolytic disorder (like sickle cell anaemia) or any known condition with shortened red blood cell survival were excluded. Also recent blood transfusion was an added exclusion criteria. Their blood samples were collected and analyzed for HBA_{1C}, as in the patient's group. The patients' ages were stratified into age groups separated by a decade, from 20-70years and above. They were also partitioned according to their sexes.

We determined those patients whose HBA_{1C} values were greater than 7.2%. At this level, the risks of diabetes complications are said to be higher[5].

Epi-info-version 6.03

Statistical analysis was conducted using the Epi-info software package version 6.03. Descriptive statistics such as means and standard deviations (SD) were calculated to compare characteristics between different categories. The student T-test was used to determine level of relationship between two mean values. The level of significance was taken at $P< 0.05$.

RESULTS

Five hundred diabetic patients and four hundred controls were included in this study. The general characteristics of the patients in this study are shown in Tables 1a and 1b. From table 1a, fifty-six percent of the study populations were females, while males made up the remaining forty-four percent. Most of the patients (>90%) were over the age of 40 years. Only four percent of the patients were below the age of forty. On the other hand, table 1b revealed that about 72% of the patients had diabetes for less than ten years. Sixty-four percent of these patients, who had diabetes for less than ten years, actually had only been diabetic for less than five years. Only ten patients (2.0%) had diabetes for more than twenty-five years. All the patients who had diabetes for fifteen years and above were females.

The result of Body Mass Index (BMI) revealed that 72(36%) male patients were overweight, while 192(64%) of the females were overweight. This showed a higher tendency towards obesity among the female patients. Only female patients had a BMI greater than $30Kg/m^2$. For most patients, the BMI was in the $20\text{-}25Kg/m^2$ range.

The mean HbA_{1C} in all the age groups of the study population was greater than 7.2%, though comparison of the various mean HbA_{1C} values by ANOVA, in the different age groups showed no significant difference (See Table 2a). The mean HbA_{1C} values for the different age groups of the control subjects are all below 7.2% (See Table 2b). For the diabetics' subjects, the mean glycated haemoglobin was 8.0% while the mean HbA_{1C} in the control was 5.2%. When these two mean HbA_{1C} values were subjected to Student t-test a P-value of 0.0001 was obtained (See Table 2c). This shows that the two mean values were significantly different.

About 64% of the diabetic subjects investigated had HbA_{1C} value greater than 7.2%. The female diabetic population had HbA_{1C} value >7.2% in 64.5% of them, while 73.7% of the male diabetics had value >7.2%. The female diabetic patients had a mean HbA_{1C} value of 7.8% (SD=1.96) against the value of 5.1% (SD=1.13) for the female control patients. Comparison of these two mean values gave a P-value of 0.0001. Similarly, the male patients and male control subjects had mean HbA_{1C} values of 8.1% (SD=1.96) and 5.6% (SD=1.00) respectively with P-value of 0.0001 (See Tables 3a and 3b).
The control subjects had a mean fasting blood glucose level of (± SD) 4.93 ± 1.09 mmol/L and the corresponding value for the diabetics was 8.6 ± 4.3 mmol/L. when these two value were compared we got a P-Value <0.05. The fasting blood glucose level and the glycated haemoglobin levels of our patients had a strong positive correlation r = 0.93.

Table 1a: **Age and Sex Distribution of the Patients.**

Age Range in Years	No of Males	No of Females	Total	Percentage of Total
20-29	20	-	20	4.0%
30-39	-	-	-	0.0%
40-49	60	80	140	28.0%
50-59	40	160	200	40.0%
60-69	40	-	40	8.0%
= 70	60	40	100	20.0%
Total	220	280	500	100.0%

This table shows the sex distribution of our patients in different age ranges, and the relative proportions (in percentages) of the different age ranges.

Table 1b: **Duration of Diabetes Illness and Sex Distribution of the Patients.**

Duration in Years	No of Male	No of Females	Total	Percentage of Total
< 5	140	180	320	64.0%
5-9	20	20	40	8.0%
10-14	40	-	40	8.0%
15-19	-	40	40	8.0%
20-24	-	50	50	10.0%
25+	-	10	10	2.0%
Total	200	300	500	100.0%

This table stratifies the patients based on the duration of their diabetes illness and their gender.

Table 2a: **_Anova for the Mean Glycated HB in Different Age Groups of the Patients.**

Age range (Years)	Glycated HB Values < 7.2% n(%)	Glycated HB Values >7.2% n(%)	Glycated HB Mean (SD)
20-29	20(100)	0(0)	6.8(1.50)
30-39	0(0.0)	0(0)	(0.0)
40-49	40(28.6)	100(71.4)	8.9(2.04)
50-59	40(20.0)	160(80.0)	8.0(2.33)
60-69	40(100.0)	0(0)	5.3(2.19)
70 +	40(40.0)	60(60.0)	8.0(0.58)

P-value = 0.894
Analysis of variance of the mean Glycated Haemoglobin (in percentages) for the different age groups of the patients.

Table 2b: **Anova for the Mean Glycated HB in Different Age Groups of the Controls.**

Age Range (Years)	Glycated HB Values < 7.2% n(%)	Glycated HB Values >7.2% n(%)	Glycated HB Mean (SD)
20-29	37(50%)	37(50%)	6.1(1.85)
30-39	72(100%)	0(0.0%)	4.9(0.11)
40-49	109(100%)	0(0.0%)	5.0(1.02)
50-59	0(100%)	0(0.0%)	-
60-69	110(100%)	0(0.0%)	5.4(0.75)
70 +	35(100%)	0(0.0%)	4.2(0.0)

P-value = 0.33
Analysis of variance of the mean Glycated Haemoglobin (in percentages) for the different age groups of the controls.

Table 2c: **Comparism of the Mean Glycated Haemoglobin Levels between the Patients and the Control Group Using Student t-test.**

Age Range (Years)	Glycated HB Values < 7.2% n(%)	Glycated HB Values >7.2% n(%)	Glycated HB Mean (SD)
20-29	37(50%)	37(50%)	6.1(1.85)
30-39	72(100%)	0(0.0%)	4.9(0.11)
40-49	109(100%)	0(0.0%)	5.0(1.02)
50-59	0(100%)	0(0.0%)	-
60-69	110(100%)	0(0.0%)	5.4(0.75)
70 +	35(100%)	0(0.0%)	4.2(0.0)

P-value = 0.33
Analysis of variance of the mean Glycated Haemoglobin (in percentages) for the different age groups of the controls.

Table 2c: **Comparism of the Mean Glycated Haemoglobin Levels between the Patients and the Control Group Using Student t-test.**

	Mean GHB	SEM	N	
Patients	8.01	0.44	500	**P-Value= 0.0001**
Controls	5.20	0.34	400	

KEY: GHB= Glycated Haemoglobin; SEM= Standard error of the mean; N= Number of subjects.

Table 3: **Comparism of the Mean Glycated Haemoglobin Levels between the Patients and the Control Group According to Sex Using Student t-test.**

3A: Females

	MEAN	SD	N	
Patients	7.8	1.96	280	**P-Value=0.0001**
Controls	5.1	1.13	180	

KEY: GHB= Glycated Haemoglobin; SEM= Standard error of the mean; N= Number of subjects.

3B: Males

	MEAN	SD	N	
Patients	8.1	1.96	220	**P-Value=0.0001**
Controls	5.6	1.00	220	

KEY:GHB= Glycated Haemoglobin; SEM= Standard error of the mean; N= Number of subjects.

DISCUSSION

The average age characteristics of the Ilorin diabetics in this study are similar to those of Nabeel *et al* [8] in 1996 and Hassan *et al* [9]. There were more females than males in our studied population. Other workers [8,10] have reported this picture of more women in the study populations . The plausible reason for this gender picture is that there is increased frequency of clinic attendance by females compared to males [9]. Also, the fact that in most developing countries men are the bread winners can account for their poor availability for clinic attendance. Most of our patients (>90%) were over the age of 40 years. Only about 4% of them were below 40 years, this is similar to a report from another third world country [10]. This is in line with the general thinking that Type II diabetes is mostly maturity in onset.

Considering duration of diabetes mellitus, seventy-two percent of our patients have had diabetes for less than 10 years. In a similar report[10], 71% of the diabetics had the disease for less than ten years. Another study[9] showed that 77% of the patients had diabetes for less than 10 years. The reasons advanced for the high percentage of patients with less than 10 years of disease duration is that, either diabetes is associated with recent changes in dietary habits and life style changes, or it is a reflection of high mortality among the patients[10]. Ilorin being a rapidly growing urban centre in a third world country (Nigeria) could easily fit in into the above reasons adduced for high percentage of patients with short disease duration. This observation could also result from the relatively low life expectancy in Nigeria coupled with the fact that majority of the patients were over forty years in age.

The patients' mean glycated haemoglobin (HbA1c) level in our study was 8.0%. This was higher than 7.2%, the level at which development of diabetic complications was commoner[5, 8, 9]. Sixty-four percent (320) of our subjects had HbA_{1C} value 7.2%. In one of the studies mentioned above[8], the mean HbA_{1C} was 9.5%[9]. In this particular study, the authors were of the opinion that such high level of HbA_{1C} was indicative of an unimpressive antidiabetic treatment among their subjects. Akbar[20] in Saudi-Arabia noted that 67% of their diabetic patients had poorly controlled blood glucose. Poor glycaemic control in their study was defined by HbA_{1C} > 7.0%. Their lower cutoff value could account for the relatively higher proportion of their patients falling into the group of poorly controlled patients. These high levels of glycated haemoglobin and the high prevalence of patients with poor glycaemic control tend to support the second proposition above, that high mortality among diabetic patients may be responsible for most study patients being recent diabetics (<10 years).

Poor glycaemic control has been linked in various studies with increased predisposition to worsened morbidity and mortality. In a Finnish study[11], it was discovered that glycated haemoglobin was the most important single risk factor associated with coronary heart disease (CHD) death or all coronary heart disease events. Furthermore, even after adjustments for other cardiovascular risk factors, HbA_{1C} was still significantly associated with coronary heart disease death.

The mean glycated haemoglobin (HbA1c) level of 8.0% in our study was significantly higher than the control mean value of 5.2% (P-value = 0.0001). This further support the thinking that the high value found in the patients was due to their diabetes and not as a result of general population characteristics in this environment. Also, it adds strength to the need to do more for our patients in terms of their glycaemic control. When the mean values were compared at gender level the significant differences were still maintained.

Microalbuminuria and macroalbuminuria have been shown to be frequently associated with increased glycated haemoglobin values[12]. Hashimoto *et al*[13] in

Japan found that prevalence of proteinuria in subjects rose significantly as HbA_{1C} level rises. In another study, it was suggested that the absence of albuminuria after 20 years or more of diabetes is associated with low HbA_{1C}[14]. Glycated haemoglobin has also been linked with ophthalmic diseases. Leske *et al*[15] showed HBA_{1C} to be positively associated with cortical and posterior subcapsular opacities of eye lens.

These varied effects of increased glycation in diabetics might not be unconnected with the evidence that glycation itself may induce the formation of oxygen free radicals. This has been linked to cholesterol peroxidation[10] in erythrocytes of diabetes, and even in healthy subjects[16].

We noted that more males had HBA_{1C} value greater than 7.2% than females in our study (P0.05). Since HBA_{1C} elevation has been linked with deleterious effects, it may therefore be that complications of diabetes with possible attendant deaths are commoner among males. This could partly explain why we have more female diabetics attending outpatient clinics. Also, our finding from this study that all the patients who had diabetes for fifteen years and above were females further supports a possible association between mortality and the observed relatively higher HBA_{1C} value in males.

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