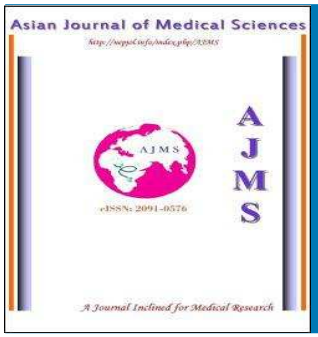

RELATIONSHIP OF GLYCATED HAEMOGLOBIN (HBA1C) AND GLUCOSE IN STREPTOZOTOCIN-INDUCED WISTAR RATS IS DETERMINED BY LINEAR REGRESSION

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[1] G.S. George, [2] A.A.Uwakwe [3] G.O. Ibeh[1] 1. Department of Medical Biochemistry, Niger Delta University, Amassoma, Bayelsa State, Nigeria 2 &3.Department of Biochemistry, University of Port Harcourt, Nigeria.

CORRESPONDENCE:

Dr.G.S. George
Department of Medical Biochemistry,
Niger Delta University,
Amassoma, Bayelsa State, Nigeria
+2348035478018
e-mail:
docgeogborie@yahoo.com

"The pattern of result would seems to compliment the fatc that in diabetes mellitus, the intracellular proteins of most tissues affected undergo both spontaneous and progressive non-enzymatic glycosylation capable of altering the activity and antigenicity of proteins"

ABSTRACT

Objective

To evaluate the relationship of glucose and glycated haemoglobin (HbA1C) in type 1 diabetes model induced by streptozotocin.

Research Design and Methods

Induction of diabetes mellitus was achieved through the intraperitoneal injection of 70mg/kg body weight of streptozotocin dissolved in 1m citrate buffer p^H 4.5 twice daily for 2 days. A total number of thirty rats were used selected among those that have exceeded glucose threshold (>10.0mmol/l) 2 weeks after streptozotocin induction. All rats weighed between 240-300g. Samples for fasting plasma glucose and glycated haemoglobin were collected at the tail vein. Glucose was determined by the glucose oxidase method and HbA1C was determined by High Performance Liquid Chromatograph (HPLC-Esi/ms) with uv detection. Data was analysed by one way and two way analysis of variance using SPSS version.

Results

Significant linear relationship was demonstrated between plasma glucose level and glycated haemoglobin which could be predictive of risk of developing diabetes. Control samples had values within reference range, glucose (3.5-6.5 mmol/l) and glycated hemoglobin (4.3-7%). However diabetic test rats elicited values that varied significantly with time. Test result confirms the fact that higher mean values of plasma glucose in diabetic (positive) controls were due to the effect of streptozotocin.

Conclusion

Plasma glucose and glycated hemoglobin show positively mutual relationship and can be used in early diagnosis of diabetes mellitus. Using correlation coefficient and regression enhances measurement of the strength of the bivariate association and is predictive.

Keywords: Glycated haemoglobin, Glucose, Streptozotocin, Regression, Correlation Coefficient.

INTRODUCTION

The use of glycated hemoglobin as a screening test for diabetes mellitus has become relevant. Its advantage has vitiated the use of other test like oral glucose tolerance (OGTT), 2-hours postprandial, Random or Fasting blood glucose. It is convenient for both the patient and the healthcare provider since samples can be collected at anytime of the day. The additional advantage being an indicator of the glycemic status over the previous 4-6 weeks is reflective of its use in management Wolffenbutted et al (1980)[1] and WHO (1980)[2] have suggested that long-term modification of hemoglobin by advanced glycosylation end products (Hb-AGEs) would be a better index for long-term glycemic patient having diabetes. The observation that AGEs are formed on hemoglobin suggest that HbA1C is a precursor for Hb-AGE, which is stable to dialysis, acid precipitation and proteolysis. Reactive derivatives from non-enzymatic glucose protein condensation reactions as well as nucleic acid and lipids that are exposed to reducing sugars notably glucose, form a heterogeneous groups of irreversible adducts called Advanced glycated endproducts (AGEs). They were earlier characterized by a yellow-brown fluorescent colour and in addition to their capacity to form cross-links with and between amino groups. AGEs are now used broadly to include advanced products of glycation process the "Maillard Reaction" which includes N- carboxymethyllysine (CML) and pyrraline which do not cross-link, neither fluorescence nor show colour Brownlee el al (1988) [3] and Takeuchi el al (2004)[4]. CML are formed from the precursors of glyoxal and glycoaldehyde by an intramolecular cannizzaro reaction, a process that is largely independent of glucose autoxidation has elucidated the importance of glycosylated hemoglobin in diabetic complications. Interestingly, the concept that CML is a marker of oxidation rather than glycation has recently attracted support. It is now known that the formation of AGEs in-vitro and in-vivo is largely dependent on the turnover rate of the chemically modified target, the time available, and the sugar concentration. The structures of the various cross-linked AGEs that are generated invivo have not yet been chemically determined, this is largely due to their heterogeneity and the complexity of the chemical reactions involved. AGEs are formed by the Milliard process, a non-enzymatic reaction between Ketone group of the glucose molecule or aldehyde and the amino group of proteins that contributes to the ageing of proteins and several pathological complications of diabetes mellitus, Glomb M. A. et. al (1995) [5], Matsumura et. al. (2000)[6] and Grandhee S. K. et. al. (1991) [7]

In hyperglycemia elicited by diabetes, this process begins with the conversion of reversible Schiff-base adducts to more stable, covalently bound Amadori rearrangement products. In course of few days or weeks, the Amadori products undergo further rearrangement reactions to form the irreversibly bound moieties known as AGEs. It is known that AGEs can also be formed from carboxyl compounds derived from the antioxidation of sugar and other metablolic pathways. In diabetes, the mechanism of glycation is increased due to glycemic stress brought about by the metabolic perturbation produced by the diabeteic state. In this study we evaluate plasma glucose and glycated hemoglobin concentration to establish a linear relationship using regression analysis.

RESEARCH DESIGN AND METHOD

Wistar albino rats aged (15-20 weeks) derived from a colony maintained at the animal house of the Department of Biochemistry, Choba Park,

University of Port Harcourt, Rivers State, Nigeria were used for the experiment. The rats weighing between 240-300g were housed in cages within a temperature of (25±2°C) and were separated into 3 groups of 10 rats each. Group 1 was control (Normal rats) injected with equivalent volume of vehicle, group 2 and 3 were induced with 70mg/kg body weight of rat with streptozotocin (Zanosar), dissolved in 1m citrate buffer p^H 4.5 for 2 days. The animals were considered diabetic when the blood glucose values exceeded 10 mmol/L 2 weeks after induction. Group 3 animals were subjected to Daonil (glibenclamide) for 16 days. Sample collection was by the modified method of Voss E. M. et. al. (1992)[8] HbA1C was measured by using an ion-exchange high performance liquid chromatography (HpLc-Esi/ms) approach with µv detection. Fasting blood glucose was measured using enzymatic oxidation method with glucose oxidase. This study was approved by the Research Ethics Committee of the College of Health Science, University of Port Harcourt, Rivers State, Nigeria.

RESULTS

Tables 1 and 2 show assay values for plasma fasting blood glucose (FBG) and glycated hemoglobin respectively for control, Diabetic test rats and rats treated with Daonil.

Fig 1 is a plot of individuals according to their HbA1C and fasting plasma glucose values. HbA1C and fasting plasma glucose were linearly related and the linear regression line had a correlation coefficient of r=0.69 significant at 0.01 level (r=0.69, p<0.01) while glucose and HbA1C levels for the controls were stable, there was marked elevation for the diabetic test rats.

DISCUSSIONS

We have studied the relationship between glycated hemoglobin and glucose using Type I diabetic models by induction with streptozotocin. Result

Table 1: Fasting plasma glucose (FPG) mmol/l assay values for the different groups

Day	Control	Diabetic control rats	DTR on Daonil
0	4.6±0.03^{a}	10.3±0.03^{b}	18.3±0.06^{c}
2	4.7±0.03^{d}	10.7±0.03^{a}	16.1±0.11^{f}
4	4.4 ± 0.03^{s}	11.4±0.00^{b}	14.2±0.11^{i}
6	4.5±0.03^{j}	13.3±0.08^{k}	12.0±0.15^{k}
8	4.7±0.03^{l}	15.5±0.05^{z}	10.0±0.22^{m}
10	5.1±0.08^{n}	16.4±0.11^{q}	9.3±0.24^{v}
12	5.3±0.08^{s}	18.2±0.08^{t}	7.0±0.27^{u}
14	4.6±0.12^{v}	22.3±0.08^{v}	5.2±0.11^{x}
16	4.7±0.08$_{v}$	24.3±0.93^{z}	4.4±0.27^{a}

Values are mean ± SEM of triplicate determination. Values on the same row having the same subscript are not significantly different from each other.

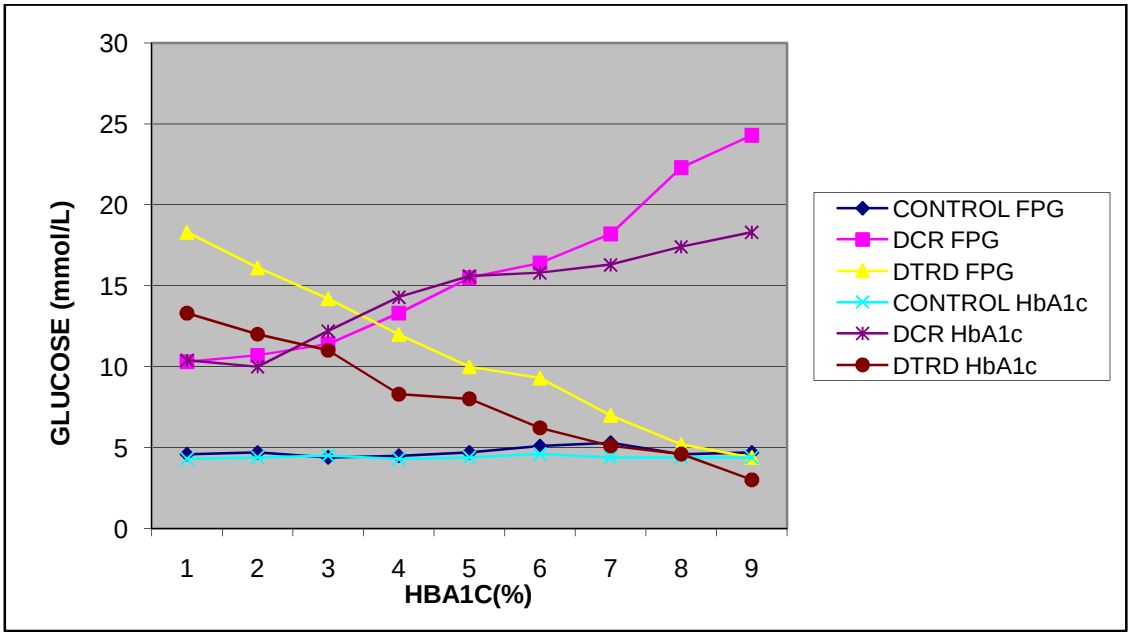
Table 2. Glycated Haemoglobin (HbA1C)% Assay Values for Different Groups

Day	Control	Diabetic control rats	DTR on Daonil
0	4.3±0.03^{a}	10.4±0.08^{b}	13.3±0.51^{c}
2	4.4±0.06^{d}	10.0±0.57^{e}	12.0±0.31^{f}
4	4.5±0.05^{g}	12.2±0.10^{h}	11.0±0.8^{h}
6	4.3±0.03^{i}	14.3±0.05^{i}	8.3±0.21k
8	4.4±0.40^{l}	15.6±0.03^{m}	8.0±0.41^{n}
10	4.6±0.03^{q}	15.8±0.35^{r}	6.21±0.11^{s}
12	4.4±0.40^{t}	16.3±0.03^{u}	5.1±0.23^{v}
14	4.4±0.30^{w}	17.4±0.02^{x}	4.6±0.22^{y}
16	4.4±0.57^{z}	18.3±0.04^{t}	3.0±0.23^{u}

Values are mean ± SEM of triplicate determination. Values on the same row having the same superscript are not significantly different from each other.

show that rising glucose levels for the induced sample was due to the streptozotocin. Although it has been recommended that screening for

Fig 1: Correlation between FPG and HbA1c in normal, diabetic and daonil treated wistar albino rats



Legend: Fasting plasma glucose (FPG) and glycated haemoglobin (HbA1c) correlated. The relationship is described by the regression equation indicated. DCR: Diabetic Control Rats, DTRD: Diabetic Test Rats on Daonil

diabetes be accomplished primarily by measuring fasting plasma glucose, fasting is inconvenient for patients couple with failure of patient to fast properly. This can result is misdiagnosis of diabetes. In contrast HbA1C can be measured at any time of the day regardless of the length of fast or content of previous meal all of which are advantages vitiated by fasting blood sugar (FBG), 2 hours post prandial glucose and even oral glucose tolerance test (OGTT). HbA1C can be analysed with a small amount of sample, as little as 5µl of blood obtained from a finger prick. HbA1C is a more comprehensive measure of total glycemic exposure than fasting blood glucose in that it is a measure of plasma glucose not only in the fasting state but also in post prandial state. Here it can be a better prediction of glycemic related complications.

HbA1C is highly correlated with the presence of diabetic microvascular complications. As further demonstrated by Mc Lance et al (1994)[9] , Sho-Chi Yamagishi et. al. (2005)[10] and Kilpatrick E. S. et. al. (1998)[11] HbA1C is as effective a predictor of microvascular complications as fasting plasma glucose. In an attempt to explain the complication of diabetes it is possible to state that the synthesis of HbA1C elicit a model reaction to elucidate the biochemical basis for many of the long term sequalae of diabetes. The tissues that suffer most noticeable dysfunction in diabetes (e.g. kidney, peripheral nerves, retina, lens) appear to be insulin dependent for glucose uptake. Studies point to the fact that in diabetes mellitus intracellular protein of these tissues undergo excess non-enzymatic glycosylation and are known to alter the enzymatic activity, solubility, antigenicity and other functions of protein which accounts for the observed clinical dysfunction. As earlier shown by Bucala et al (2003)[12], Naka et al (2004)[13] and Vlassra (2005)[14], there is increasing evidence to support that inhibition of advanced glycated end products (AGEs)

or blockade of their down stream signaling pathway could be a promising strategy for treatment of patients with diabetic complication.

We have used correlation coefficient to measure the strength of the bivariate association of HbA1C and glucose and with regression to predict values of HbA1C for a given glucose concentration.

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