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

**LINEAR REGRESSION**

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**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 pH 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) 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 haemoglobin 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 (BrownLee M. et al, 1988). 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 (Takenhim M. et al 2004). CML are formed from the precursors of glyoxal and glycoaldehyde by an intramolecular cannizzaro reaction, a process that is largely independent of glucose autoxidation (Glomb M.A., et al 1995). 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 invitro and invivo 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 adehyde and the amino group of proteins that contributes to the ageing of proteins and several pathological complications of diabetes mellitus. (Matsumura et al 2000, Sho-Ichi et al 2005).

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.

**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+2oC) and were separated into 3 groups of 10 rats each. Group 1 were controls (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 pH 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. 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.

**RESULT**

Tables 1 and 2 show assay values for plasma fasting blood glucose (FBG) and glycated haemoglobin 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.

**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+0.03a | 10.3+0.03b | 18.3+0.06c |
| 2 | 4.7+0.03d | 10.7+0.03a | 16.1+0.11f |
| 4 | 4.4 + 0.03s | 11.4+0.00b | 14.2+0.11i |
| 6 | 4.5+0.03j | 13.3+0.08k | 12.0+0.15k |
| 8 | 4.7+0.03l | 15.5+0.05z | 10.0+0.22m |
| 10 | 5.1+0.08n | 16.4+0.11q | 9.3+0.24v |
| 12 | 5.3+0.08s | 18.2+0.08t | 7.0+0.27u |
| 14 | 4.6+0.12v | 22.3+0.08v | 5.2+0.11x |
| 16 | 4.7+0.08v | 24.3+0.93z | 4.4+0.27a |

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

**2: Glycated Haemoglobin (HbA1C)% Assay Values for Different Groups**

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

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

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

DCR FPG = 1.57 DCR HbA1c – 6.905

DTRD FPG = DTRD HbA1c 1.344 + 0.041

Control FPG = 1.113 Control HbA1c – 0.176

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

**DISCUSSION**

We have studied the relationship between glycated haemoglobin and glucose using Type I diabetic models by induction with streptozotocin. Result show that rising glucose levels for the induced sample was due to the streptozotocin. Although it has been recommended that screening for 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) 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 Bucarelli et al (2003), Naka et al (2004), Vlassra (2005), 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.

**REFERENCES**

Brownlee M, Cerami A., Vlassara H. (1988) Advanced glycosylation end

products in tissue and the Biochemical basis of diabetic complication. N.Engl 318:1315-21

Bucala R., Vlasara H., (1995) Avanced glycosylation end products in

diabetic renal and vascular disease. Am J. Kid Ais 26:875-888

Glomb M.A., Monier V.M., (1995) Mechanism of protein modification by

glyoxal and glycoaldehyde, reactive intermediates of the milliard reaction. J. Biol chem. 70:10017-26

Grandhee S.K., Monier V.M., (1991) Mechanism of formation of Milliard

protein cross-link pentosidine. Glucose, fructose, and ascorbate as pentosidine precusros. J. Biol Chem. 266:11649-53

Kilpatrick E.S., Maylor P.W., Keevil B.G., (1998). Biological variation of

glycated hemoglobin: Implications for diabetes screening and monitoring. Diabetes Care 21:261-264.

Matsumura, Yamagishi S., Brownlee M., La Leroith D., Taylor S.I.,

Olefsky J.M. Ed (2000). Advanced glycation end products and the pathogenesis of diabetic complications. Diabetes mellitin: A fundamental and clinical Text. New York, Lippincott-Raven Publishers, 983-91.

Mc Cance D.R., Hanson R.L., Charles M.A., Jacobsson L.T., Pettit D.J.,

Bennet P.H., Knowler W.C., (1994). Comparison of tests for glycated hemoglobin and fasting and two hours plasma glucose concentrations as diagnostic methods for diabetes. B.M.Y 308:1323-1328

Sho-Ichi Yamagishi, Kazuo N., Tsutomu L., (2005). Advanced glycated

end products (AGEs) and diabetic vascular complications. Curr Diabetes Review 1,93-106

Takeuchi M., Yamagishi S., (2004). Alternative routes for the formation

of glyceraldhyde-derived AGEs (TAGE) *in vivio*. Med. Hypotheses. 63:453-5

Takeuchi M., Monier V.M., Sasaki N., et al (2004). Involvement of

advanced glycation end products (AGEs) in Alzyheimers disease. Curr Alzheimer Research. 1:39-46

Voss E.M., Cembrowski G.S., Clasen B.L., Spencer M.L, Ainslie M.B.,

Haig B., (1992). Evaluation of capillary collection system for HbA1C specimens. Diabetes Care 15:700-701.

Wollfenbuttel B.H., Giordano D., Found H.W., Brucalla R., (1980). Long-

term assessment of glucose control by hemoglobin-AGE measurement. Clin Lab Haematol. 2:129-138.

World Health Organisation (1980). WHO Expert Committee on Diabetes:

Second Report. Geneva, (Tech Rep. Ser., No.646).