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EVALUATION OF REACTIVE GLUCOSE BY-PRODUCTS DICARBONYLS AND GLYCATED HAEMOGLOBIN IN HYPERGLYCEMIC PATIENTS

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ABSTRACT

To examine the effect of hyperglycemia on production of glucose by-products, we determined the concentration of glyoxal, methylglyoxal along with glucose, insulin and glycated haemoglobin (HbA1c). Using radioimmunoassay, chromatographic and spectophotometric techniques, the interactions of these compounds have been used to elucidate a possible cause of clinical significance in the diabetic state. Our study has shown that the selected analytes interacts in uncommon ways to exercerbate glycemic stress. We observed a positive linear relationship between glucose concentration and glycated haemoglobin and identified glucose excess as being a responsible factor controlling the creation of glucose by-products. Concomitant with the knowledge of the fact that both compounds are detoxified by the activity of their respective glyoxylase, we observed glyoxal concentration 3-4 folds higher than methylglyoxal in patients investigated. Further analysis of data with a scatter plot of subjects according to their blood glucose and HbA1c, glyoxal and methyloxal shows a positive linear correlation and the linear regression had a coefficient of r=0.820 significant at $P > 0.05$. The biochemical evidence suggests that production of carbonyls increases glucose toxicity and imposes the need to provide substances that will inhibit their formation. This is expected to enhance diabetes mellitus management.

Keywords: Reactive Glucose, dicarbonyls, glycated haemoglobin, hyperglycemic.

INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disorder characterized by chronic hyperglycemia due to dynamic interactions between varying defects of insulin secretion and action. The role of glucose in the pathogenesis of diabetic complications has attracted scientific scrutiny raising questions as to whether glucose is a reactant or an inert molecule in the pathogenesis of diabetic complications.

The study of glucose by-products notably glyoxal, methyglyloxal, dimethylglyoxal and 3-deoxyglucosone has attracted tremendous attention in recent years on account of their clinical significance in chronic and age related diseases as shown by Thornally *et al.* (1999). Oxidative stress is now known to be a feature of these diseases notably diabetes mellitus in which intracellular hyperglycemia in insulin dependent cells, such as nerves, kidney, lens and erythrocytes modulates the genesis of microvascular complications due to the production of advanced glycation end products (AGEs) (Dalle-Donnea *et al*. (2003) and Brownlee (2005). Further examination of periodicals by Maritin *et al*. (2003) and Brownlee (2001) has shown that these processes occur via non-enzymatic glycosylation of both intracellular and extracellular matrix proteins like collagens and extraceullular matrix proteins which inhibits nitric oxide production. The result of these stress induced metabolic activities is the modification of lipids, proteins and carbohydrates which are expressed in the generation of reactive carbonyls compounds such as glyoxal, methylglyoxal, dimethylglyoxal and 3-deoxyglucosone.

Previous scientific studies have shown that reactive carbonyl compounds are glycolytic mediators of reactive carbonyl stress which have been implicated in diabetes (Saka, 2011). It has earlier been shown that the Milliard reaction (Advanced glycated end product) exacerbate protein glycation, raises synthesis of reactive oxygen species (ROS) and metal ions which have been involved as participants in complications of diabetes. Oxygen is now known to be a fixative of irreversible damage via this advanced glycated end product reaction.

Our understanding of recent studies have elucidated the fact that advanced glycated end products of the Milliard reaction can be inhibited as reflected in Brownlee *et al*. (1986) and Payrox and Sternberg (2006). The vitamin B_6 pyridoxamine is considered an Amadorin or post Amadori inhibitor, which is known to trap products from Amadori compound fructoselysine, the first stable glucose aduct protein. The other significant roles played by pyridoxine includes blocking oxidation, trapping reactive carbonyl and dicarbonyl compounds, chelation of metal ion catalyst and scavenging of reactive oxygen species as shown by

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Khalifa *et al*. (1999), Reddy and Beyaz (2006) and Jomova *et al.* (2010). Some authors have shown other carbonyl trapping agents with reactive nucleophilic functional groups invitro as expressed in rodent models of diabetes. These compounds include 2,3-diaminophenazone, penicillamine and several derivatives of aminoguanidine.

In this work we have studied some of the metabolites which are very important in diabetic complications.

MATERIALS AND METHODS

At the Federal Medical Centre, Yenagoa, Bayelsa State, Nigeria, blood and urine samples were collected from hyperglycemic patients (n=80) with Fasting blood glucose value ≥20.0mmol/l and urine glucosuria. Control subjects (n=60) with Fasting blood glucose level ranging between 3.5-6.7 mmol/l without glucosuria were included.

Analytical Methods

The Fasting Blood Glucose was determined by the glucose oxidase method using Randox Kits and values measured with spectrophotometer set at 540nm wavelength. Urine samples were evaluated for the presence of glucose with the aid of N-multistix manufactured by Macherry-Nagel (Germany). Glycated Haemoglobin was determined by ion-exchange high performance liquid chromatography, HPLC-Esi/ms approach with UV detection. Radioimmunoassay was used for the determination of insulin with the aid of Cx 9 Automated Machine (Beckman) with Beckman assay kits.

Glyoxal and methylglyoxal were measured by the analytical method developed by Merc making use of stillbenediamine (SD) as derivating reagent at a separation time of 5 minutes and SDS as micellular medium at pH 8 and sodium tetraborate (0.1m) as buffer.

RESULTS AND DISCUSSION

Assay of the biochemical parameters determined are shown in tables 1 and 2. The results of the study gave consistent proof of the fact that the degree of hyperglycemia positively strongly correlates with the concentration of HbA1c, glucose, glyoxal and methylglyoxal and a negative correlation with insulin level. We have shown in figure 1 graphically the interrelationship of glycated haemoglobin in intact and lysed cells. Figures 2, 3 and 4 are scatter plots of some parameters determined.

Evidence of the fact that the development of complications in diabetics is strongly connected with the invivo surrounding factors such as genetics and excess production of oxygen free radicals have been proven. The determination of concentration of Fasting blood glucose, glycated Haemoglobin, insulin and the carbonyl compounds were deployed in this study to further demonstrate the influence of these parameters in diabetes mellitus. It is known that diabetes is associated with chronic complications such as macrovascular (cerebrovascular and coronary artery disease) and microvascular (nephropathy, neuropathy and eye disease). This work confirms the fact that a greater percentage of diabetic patients have one form of complications or the other at the time of diagnosis.

Table 1. Comparative values of measured profiles.

Parameters	Control (n=60)	Hyperglycemic (n=80)	P-value (≥ 0.050)
FBG (mmol/l)	4.8 ± 1.2	25.49 ± 0.48	
Insulin (iu/mol)	5.3 ± 0.2	0.18 ± 0.003	
HbA1c (mmol/mol)	42 ± 5	150 ± 6.7	
GO (ng/ml)	0.18 ± 0.12	0.54 ± 0.03	
MGO (ng/ml)	0.03 ± 0.4	0.19 ± 0.009	

Values are mean ± SD

Table 2. Determination of levels of glycated haemoglobin intact and disrupted cells.

Intact cells		Disrupted cells	
HbA1c (mmol/mol)	Glucose (mmol/l)	HbA1c (mmol/mol)	Glucose (mmol/l)
35.0	5.0	48.0	4.7
35.0	5.0	42.0	4.0
35.0	5.0	53.0	8.0
39.0	6.0	75.0	10.0
40.0	7.0	85.0	15.0
40.0	7.0	88.0	20.0
42.0	8.0	92.0	25.0
47.0	10.0	96.0	30.0

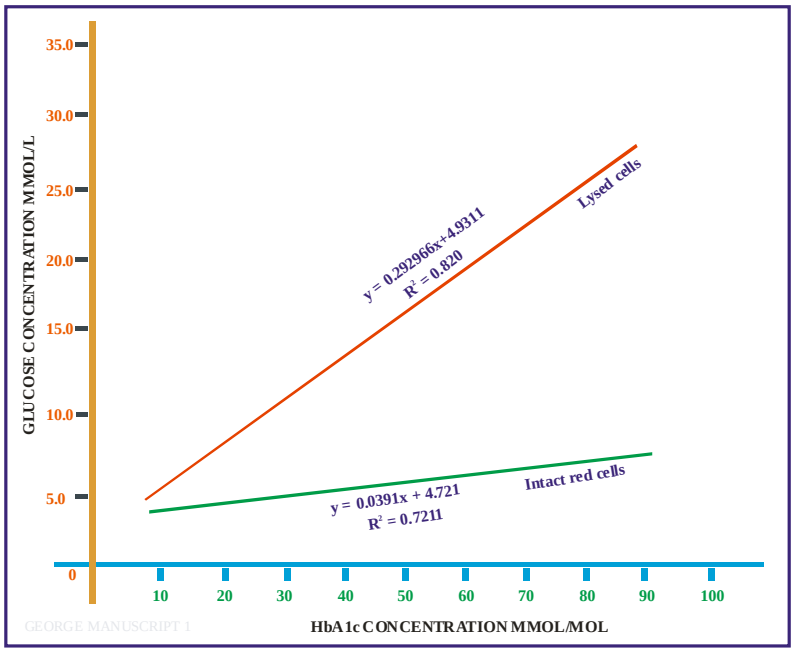

Fig 1. Graph of FBG against HbA1c (lysed and intact).

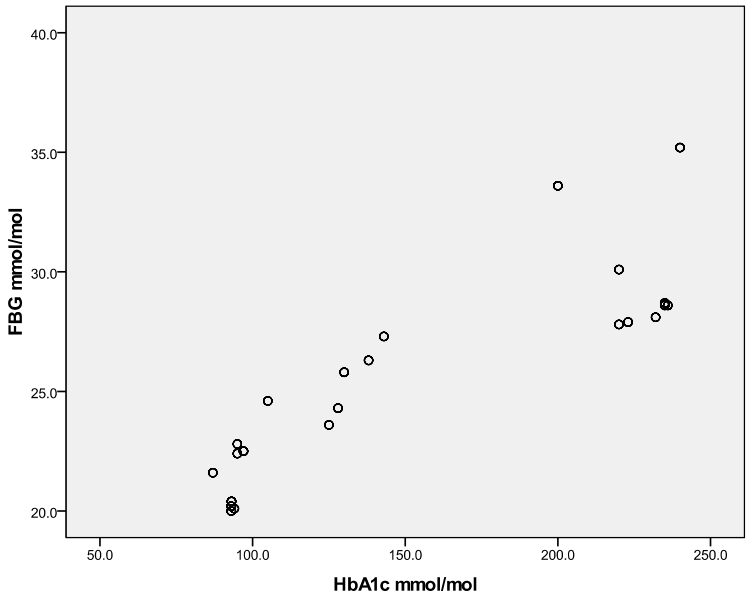

Fig. 2. Scatter plot of FBG against HbA1c.

Earlier reports showing that strong electrophilic carbonyl compounds notably glyoxal, methylglyoxal and 3-deaoxyglycosone produced in hyperglycemics are responsible for the observed glycemic stress in diabetes mellitus has been demonstrated by Odeti *et al*. (1999). Carbonyl groups result from protein oxidation and their level in tissues and plasma is a relatively stable marker of oxidative damage. Although the synthesis and eventual release of these compounds may be insidious, their sustained generation progressively causes glycation of protein which are known to contain both acidic and basic groups. A major consequence of glycation is the alteration of functional properties of proteins as elucidated by Giaco and Brownlee (2010). The elevated levels of glucose, glycated haemoglobin and the carbonyls, glyoxal and methylglyoxal observed in this study correlates with earlier findings suggesting that a major cause of lipid peroxidation is carbonyl stress. It is known that oxidative stress modulates lipids, protein and carbohydrate in ways that enhances the production of carbonyl compounds.

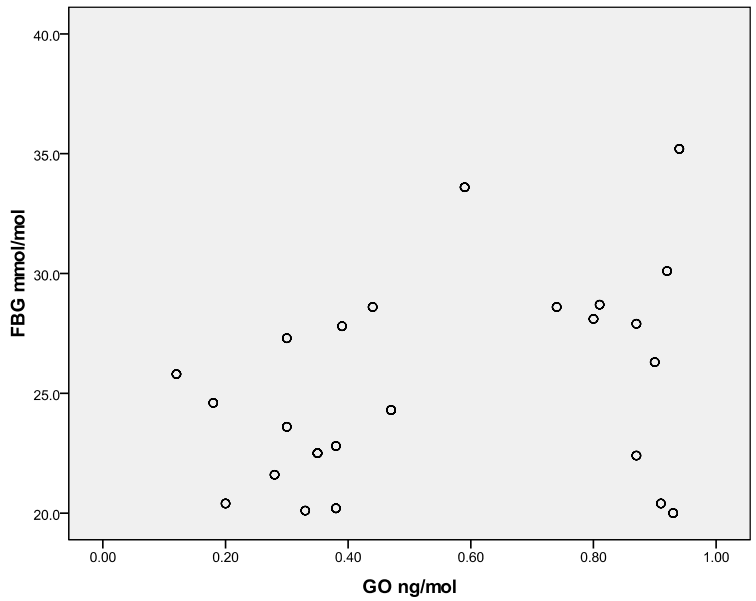

Fig. 3. Scatter plot of FBG against GO.

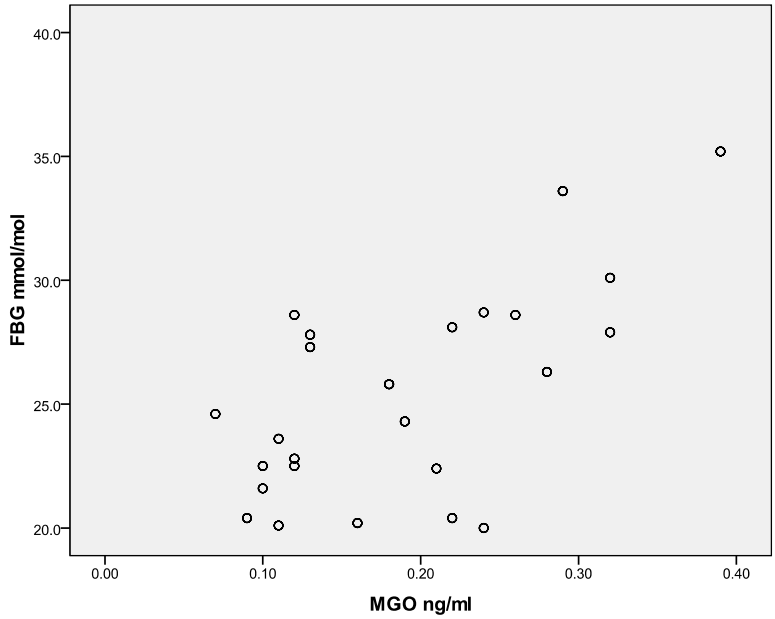

Fig. 4. Scatter plot of FBG against MGO.

Other pathways through which carbonyl could be formed are available by mechanisms which modify proteins leading to development of activated glycated end product like pentosidine and carboxymethyllysine which are known to crosslink protein (Baynes,1991; Thornally, 2004; Goh and Cooper, 2008). Recent periodicals of Riboulet-Charvey *et al*. (2006) and Pennathir *et al*. (2005) have elucidated the fact that methylglyoxal can cause inhibition of insulin stimulated phosphorylation of kinase, a factor known to have a negative effect in the insulin-stimulated phosporylaton of protein kinase with direct inhibitory role of insulin in the induced phosphorylation of substrates. The hormone insulin is a major vehicle for intracellular control and overrides the normal cellular controls. The complex nature of carbonyls compounds which are formed as a result of complications of diabetes could further act to synergise the non-enzymatic reaction that precipitates glycated end products. This may lead to receptor binding of the peptide and further exacerbate hyperglycemic condition. This view seem to lend

credence to earlier works of Zythen *et al*. (2008), Thornally (2008) and Whiting *et al*. (2008).

To further buttress the influence of glycation, we measured the glycation of haemoglobin in lysate and intact red blood cells and confirmed higher values in the lysate. A correlation coefficient determined for this gave a value of $y=0.292966x + 4.9311$ $R^2=0.820$ for the lysate and $y=0.0391x + 4.721$, $R^2=0.7211$ for the intact cells.

We observed a close relationship of the carbonyl concentration and the level of hyperglycemia which established the fact that they can be used as stable markers of oxidative damage. HbA1c, a marker of glycemic control was also related to the level of carbonyl. These results strongly suggest that impairment of glycemic control has link with oxidation. This is further buttressed by the fact that glycation cascade also releases free radicals becoming responsible for further oxidative attack which supports earlier studies of Pinaki *et al.* (2010), Tomic *et al.* (2013) and Konukoglu *et al*. (2002). The fact established here is that increased oxidative stress if any in the diabetic group is undoubtedly induced by hyperglycemia.

CONCLUSION

This work has identified some factors implicated in the production of toxic glucose by products and underscores the need for effective glycemic control which is required to prevent complications. The need to develop drug therapy which would act as inhibitors of signaling compounds for activated glycated end product formation may eventually prevent production of carbonyls and reduce the associated toxicity while providing glycemic stress reducing antioxidants.

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