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Original Research Article

HYPERGLYCEMIA RELATED OXIDATIVE STRESS: BIOMARKERS IN TYPE 1 AND2 DIABETES MELLITUS

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Abstract: The production of reactive oxygen species resulting in oxidative stress due to formation of free radicals is now known to be a feature of many diseases including diabetes mellitus. In this study, we have used some biomarkers of oxidative stress, TBARs, SOD, GSH-Px, HbA1c, glyoxal and methylglyoxal along with some other parameters, glucose and insulin to objectively estimate and analyse the condition as indicators in the control (normal) subjects and in patients with pathologic disease process of type 1 and 2 diabetes. Applying kinetic, spectrophotometric, radioimmunoassay and chromatographic methods we have assayed the indicator biomarkers. Results obtained demonstrate the fact that higher increases in the concentration of the analytes up to 3-fold were found in type 2 diabetics in the concentration of glyoxal, methylglyoxal, TBARs, HbA1c at $p=0.005$, $p>0.05$, $p=0.002$ and $p=0.003$ respectively when compared with the control. There were, however a moderate decrease in the concentration of S0D and GSH-Px. We summaries that a combination therapy for the oxidative stress management to reduce TBARS, HbA1c and the dicarbonyls can inhibit further complications in diabetics.

Keywords: *Biomarkers, hyperglycemia, oxidative stress, Type 1, Type 2 Diabetes.*

Introduction

Evidence of the global epidemiology of diabetes mellitus elucidates a dangerous trend with a rising morbidity and mortality transcending both young and elderly. Three main types of diabetes are known, type 1, type 2 and gestational diabetes. Type 1 is now known to be caused by an autoimmune reaction in which the body's defense system attack the insulin producing cells of the pancreas inhibiting the production of insulin required by the body for carbohydrate metabolism. Risk factors of type 1 may include autoimmune, genetic or environmental factors (Delmastro and Piganelli, 2011). Earlier evidence of oxidative stress in type 1 diabetes mellitus has been shown by Kaneto, *et al* (2007), Watson and Lowath (2009) in which it was observed that a cause and committed step in type 1 diabetes entails the reduction in β-cells as a

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result of the activation of pro-apoptic signaling event. There is now evidence to show that pancreatic β-cells are sensitive to cytotoxic derangement brought about by reactive oxygen specie as gene expression and activity of antioxidant enzymes, especially glutathione peroxidase action is reduced in cells, Lenzen *et al* (1996). The mediation of the diversion of glycolytic intermediates into pathological pathways occasioned by the role of oxidative stress in vascular endothelial cells has had scientific evidence as shown in Rolo and Palmeira (2006); Turk (2010).

The production of oxygen free radicals and a reduction in antioxidant defense mechanism are known to exacerbate oxidative stress. In diabetes free radicals are formed disproportionately due to glucose oxidation, degradation of glycated protein and non-enzymatic glycation of protein (Rodino-Janeiro *et al*, 2010). In type 2 diabetes, the predominating factors are genetic, characterized by an absence of particular human leukocyte antigen (HLA) associations, a non-relationship to viral infections and an absence of inflammatory cells in the islets and a lack of circulating islets cells antibodies. A striking hallmark of type 2 diabetes mellitus is the presence of obesity and insulin resistance concomitant with insulin resistance, there is a selective impairment in the β-cells response to glucose since response to non glucose secretagogues have been noted. Arising from the above factor, there is usually a reduction in the pancreatic β-cells numbers while the ones that survives have impaired secretory ability with evidence of genetic heterogeneity. It has been suggested that the development of oxidative stress through a single unifying mechanism of superoxide production, springs a common pathologic factor leading to insulin resistance, β-cells dysfunction, impaired glucose tolerance whose end product is the development of type 2 diabetes mellitus (Ceriello and Motz, 2004). Nutritional factors relating to excess glucose and fatty acid over abundance and a sedentary life style necessitates accumulation within muscles, pancreatic cells and adipose tissues resulting in generation of excess reactive oxygen species notably superoxide anion via the mitochondrial electron transport chain (Brownlee 2001; Madduxe *et al*; 2001). As earlier suggested by Hirsh (2005) unsteady glycemic state such as those observed in post-prandial glyceamic excursion in people having impaired glucose tolerance or type 2 diabetes mellitus complicates the condition. Some invitro studies have been able to show how increased levels of markers of oxidative stress in exposed cells are expressed. Inflammation, a well known marker of oxidative stress have also been observed as a response to elevated glucose concentration (Hirsh, 2005).It has been noted in a study which compared the effect of inconsistent against constant glycaemic condition done on cultured human kidney cells that the release of the inflammatory cytokines, insulin like growth factor binding protein (1GFBP)3, and transforming growth factor β(TGF-B) were evidently increased when exposed to variable glucose concentration compared with constant hyperglycaemic conditions. The authors concluded that while maintenance of normal blood glucose would result in smallest degree of oxidative stress and inflammation in the tubuloinstestitial, variable glycaemic control would likely be even more damaging than constant hypoglycaemia(Santilli, 2004). The cardiovascular system is not also sparred by the diplitating effects of oxidative stress as there is usually pronounced effect on nitric oxide availability, inflammatory response and lipid and lipoprotein modifications (Basta *et al*, 2004). We have investigated the levels of some biomarkers : Glycated Haemoglobin (HbA1c), Thiobabituric acid-reacting substance (TBARs), Superoxide Dismutase (SOD), Glutathione peroxide (GSH-Px) along with insulin levels, glucose, methylglyoxal and

glyoxal in type 1 and 2 diabetic patients using non diabetics as controls.

Materials and Methods

At the Federal Medical Centre, Yenagoa, Bayelsa State, Nigeria, a total of 320 subjects participated in this research after getting ethical approval from the Ethical Committee. Out of the subjects 110 were type 1 diabetic patients and another group of 110 were type 2 diabetic patients. Another group of 100 subjects were normal subjects with no evidence of diabetes. Selection of subjects were based on previously determined Fasting blood glucose and insulin levels.

The diabetics type (1 and 2) had glucose level above threshold (>10.0 mmol/L) with glucosuria while the non-diabetes had glucose levels ranging between 4.5-5.5 mmol/L and no glucosuria. Serum glucose (mmol/L) was measured with commercially prepared kit, Agape, a product of Agape Diagnostics (Switzerland GmbH) in a Cobas autoanalyser . Glycated haemoglobin (mmol/L) was determine by chromatographic method, utilizing a procedure that measures the glycated haemoglobin fractions HbA1a, HbA1b and HbA1c adopting ion exchange high performance liquid chromatography HPLC-Esi/ms approach with UV detection using the algorithms to obtain the value for HbA1c:, HbA1c=HbA1-(0.14/1.23) (DCCT, 1995).

To determine the activities of glutathione peroxidase and superoxide dismutase the washed cells fractions were utilized as per the kinetic method of Amstrong *et al* (1992) and the units of activity normalised per milligram of haemoglobin. The method described by Ohkawa *et al* (1979) a lipid peroxidation assay was used for the determination of thiobarbituric acid-reactive substance (TBARs). The supernatant of tissue homogenate (200µl) was added to 100µl sodium dodecyl sulphate (SDS). This was mixed with 750µl of 20% acetic acid (pH 3.5), 750 µl of 0.6% thiobarbituric acid and 30 µl of distilled water and incubated at 95^{o}C for 60 minutes. The samples were allowed to cool at room temperature. Thereafter 2.5ml of butanol pyridine (15:1) and 500µl of distilled water were added. It was then vortexed and centrifuged at 200g for 15 minutes. With the aid of a spectrophotometer (spectronic 22D^{+}) set at 537nm and utilizing 1,1,3,3-tetraethoxy propane as a standard, the absorbance of 3ml of the coloured layer was taken. Insulin levels were assayed by radioimmunoassay using LG Gamma Counter with Scintilattor measuring the activity of radioactive iodine in a way that decreasing pulse count rate as a function of increasing substance concentration according to the equation: lin (β/βovs log (conc).

In measuring the levels of glyoxal and methylglyoxal we adopted the analytical method by Merc using stillbenediamine (SD) as a derivating agent at a separation time of 5 minutes and SDS as micellular (0.1m) as buffer. Urine samples were determined for glucosuria with the aid of N-multistix a product of Macnerry-Nigel, Germany.

Results

The results of this study conducted to observe changes in biomarkers of both type 1 and 2 diabetes mellitus are shown in tables 1 and 2. Table 1 presents the values obtained for TBARS (mmol/l) SOD (µ/mg/Hb), GSH-Px (µbHb), Hba1C(mmol/l). In table 2 the values obtained for FBG (mmol/l), insulin (iµ/mmol), glyoxal (ng/ml) and methylglyoxal ng/ml are presented.Elevated values were observed for TBARS, HbA1c, glyoxal and methylglyoxal when compared with the control or non-diabetic samples. Values of GSH-Px, SOD and insulin however correlated negatively with TBARS, HbA1c, glyoxal and methylgloxal.

Statistical analysis,Results of triplicate determination were expressed as mean $\pm$ standard error of mean. Two way analysis of variance was used with a statistical value of $p<0.05$ considered significant.

Table 1: Values obtained for TBARs, SOD, GSH-Px, urine glucose and HbA1c for both normal (controls) diabetic and non-diabetic subjects.

	Normal	**Diabetes type 1**	**Diabetes Type 2**
TBARs (mmol/l)	2.23±0.64(110)	2.55±1.5(58) 2.33 ± 1.21 (52)	3.2±1.6(16) 3.7 ± 1.84 (42)
SOD (u/mg/Hb)	13.8±4.0 (110)	11.6±2.3(68) 13.2±3.5(42)	9.8±1.5(68) 7.1±1.8(42)
GSH-Px (u/g/Hb)	63.4±15.4(110)	50.2±12.4(68) 38.1±9.7(42)	46.7±14.3(68) 33.4±10.2(42)
HbA1c mmol/l	4.3±1.2(110)	13.5±2.1(68) 15.2±1.1(42)	15.3±1.46(68) 18.2±2.3(42)
Urine glucose	Nil	++	++

Values are means SD ± standard error of mean of triplicate determination

Table 2: Values obtained for glucose, insulin, glyoxal and methyl glyoxal for controls (normal) and diabetic subjects.

	Normal	**Diabetics type 1**	**Diabetics Type 2**
Glucose (mmol/l)	4.5±1.2 (110)	15.7±5.2(68) 20.4±4.0(42)	18.3±4.2(68) 28.1±4.4(42)
Insulin(iμ/mol)	5.6±0.3(110)	0.8± 2.5(42) 1.2 ± 1.5(68)	2.7±2.3(68) 5.4±2.5(42)
Glyoxal(ng/ml)	0.17±0.11(110)	0.23±0.14 (68) 0.61±0.2(42)	0.32±0.15(68) 18.2±0.91(42)
Methylglyoxal(ng/ml)	0.07 ± 0.5 (110)	0.17 ± 011 (68) 0.5 ± 0.14 (42)	0.20 ± 0.1 (68) 1.1 ± 0.2 (42)

Values are mean SD ± standard error of mean of triplicate determinations

Discussion

The need to understand the connection between reactive oxygen species, diabetes mellitus and associated complications informed this study. Several in-vitro studies have demonstrated elevated level of biomarkers of oxidative stress especially in mammalian cells that are not prevented from changing glucose concentration that sought to clarify the molecular mechanism through which oxidative stress exacerbate diabetic complications.

In our effort at linking both type 1 and 2 diabetes, hyperglycemia and oxidative stress, we studied the concentration of some identified markers notably TBARs, SOD, HbA1c, glyoxal and methylglyoxal. We observed increased levels of TBARS, HbA1c, and the dicarbonyls glyoxal and methylglyoxal. We observed a decrease in the levels of SOD and GSH-Px in the diabetic patients in both type 1 and 2 when compared with the control. As shown by the results in tables 1 and 2 we found a significant association between type 1 and 2 diabetes in terms of level of glucose concentration. However the level of severity as measured from the markers show that type 2 diabetes mellitus elicited higher values suggesting the potentials for increased complications.

A disequilibrium in the oxidant/antioxidant status is encouraging factor in formation of oxidative stress once the generation of reactive oxygen increase to the point of overwhelming the body's antioxidant capacity and defence. As earlier shown by (Tam *et al* 2013; Baynes, 1992; Bayness and Thorpe 1999), if free radicals are not disposed by the cellular antioxidants they could attack and damage lipids, carbohydrates, proteins and nucleic acid.

It is now known that oxidative stress plays very active role in the progression and pathogenesis

of secondary diabetic complications which inhibit insulin action. The concentration of insulin was lower in both type 1 and 2 diabetics when compared with the normal (control). Earlier workers West(2000) have shown that oxidative stress induced by reactive oxygen and nitrogen species have been proven to be critically involved in the impairment of β-cells function and facilitates the pathological process in diabetes mellitus. It is now known that islet β-cells are easily susceptible to oxidative stress on account of the reduced levels of endogenous antioxidants. An added reason is attributable to the fact that cell metabolism and potassium (Adenosine-5- triphosphate) channels in β-cells are veritable targets for reactive oxygen species and other oxidants. Once changes have occurred in the channels a consequent result is induction of dysfunction since genetic ablation of potassium (Adenosine-5-triphosphate) reduces the effects of oxidative stress on β-cells (Drews *et al* 2010).

The production of reactive oxygen species in diabetes stems from a variety of sources which are both mitochondrial and non-mitochondrial in origin. The molecular mechanism of reactive oxygen specie caused by hyperglycemia is now adduced to four major pathways which are increased polyol pathway (also known as the sorbitol-aldose reductase pathway), elevated hexosamine pathway flux, activation of protein kinase C, and increased advanced glycated end product. The constituents of reactive oxygen species includes superoxide anion (O_2-*), hydrogen peroxide (H_2O_2), hydroxyl radicals (*OH), singlet oxygen, nitric oxide and peroxynitrate. Chon *et al* (2005) and Firoozrai *et al* (2007)have shown that most free radicals are produced at low concentration during normal physiological conditions in the body and are scavenged by endogenous enzymatic and non-enzymatic antioxidants system which includes superoxide dismutase, glutathione peroxidase, catalyst as well as vitamin C and E.

From the results obtained, we can deduced that persistence of hyperglycemiais responsible for the production of oxidants lipid peroxidation including dicarbonyl. It is known that the activity of fatty acyl coenzyme increase as a result of decreased insulin in diabetes mellitus. This process causes the initiation of β-oxidation of fatty acids which consequently result in lipid peroxidation. As shown by Miyata *et al* (1999) and Witko-Sarsat *et al* (1996), the formation of carbonyl group is seen as an early and stable marker for protein oxidation as diabetes mellitus is linked with carbonyl stress. Our work supports this view as shown from the values obtained for glyoxal and methylglyoxal. There was an increase in the concentration of these reactive carbonyl compounds attributable to their enhance formation, a decreased degredation, excretion or a combination of all these factors.

The formation of advanced glycation end products such as pentosidin and carboxymethyllysine and other advanced oxidation protein products causing damage to a number of biologically important compounds have been reported by Astaneie *et al* (2005) and Telci *et al* (2000). There are now data in patients with type 2 diabetes mellitus that made evident the existence of increased oxidative stress in response to postprandial hyperglycemia since reactive oxygen species production can now be measured by nitrotyrosine Quagliaro *et al* (2003).

Our findings gives credence to the fact that while maintenance of normal glucose level will elicit a minimal degree of oxidative stress, changes in glycaemic control may also impose damaging effects. Overproduction of ROS is an important mechanism underlying the pathogenesis of diabetes mellitus. We now know that dysregulation mediated by hyperglyceamia has the capacity to enhance oxidative stress. Findings by Quagliaro *et al* (2003) using preclinical mouse models had focused on several strategies including the glutathione peroxidase-1 (GPX1) mimetic ebselen, novel activators of the transcription factor (Nrf2) acid peptide memetics to improve Nitric oxide

bioavailability. It is envisaged that knowledge of the ebselen and second generation functionality which presents as superior analogous stem from detailed investigation into the role of GPX1 in diabetes associated complications. More recent studies by Scheikote *et al* (2003) are showing the benefits of targeting the transcriptional factor (Nrf2) which is a central regulator of phase 2 detoxifying enzyme to control inflammatory related diabetic complications.Increased glucose levels responsible for hyperglycaemia is capable of manifesting a range of persistent changes such as epigenetic modification compositional changes and cellular adaptation and resetting of equilibra that contributes to the complication of diabetes.

In conclusion we summarise that as a strategy to lessen the burden of diabetes complications, treatment models that would counteract the effect of the biomarkers in this study be adopted. This will check dna damage,oxidation of polysaturated acids, oxidation of amino acids and deactivation of some specific enzymes that have implication for monitoring, prevention and development of therapeutic choice for type 1 and 2 diabetes mellitus.

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References

1. Amstrong, D., Abedela, N., Salmon, A., Miller, A.N., Rahman, E.A., Bejancy, Z.K.M. (1992). Relationship of lipid peroxidation to diabetic complications: compares with conventional laboratory test by diabetes complication 6:116-122.
2. Astaneie, F., Afshari, M., Mijtahedi, A., Mostafalau, S., Zammri, M.J., Larigani, B., Abdollahi, M. (2005). Total antioxidant capacity and levels of epidermal growth factor and nitric oxide in blood and saliva of insulin-dependent diabetic patients. Arch Med. Res vol. 36, pp. 376-381.
3. Basta, G. Schmidt, A.M., Decaterira, R. (2004). Advanced glycation end products and vascular inflammation: implications for accelerated artherosclerosis in diabetes. Cardiovasc Res. 63: 582-92.
4. Baynes, J.W. (1992). Role of oxidative stress in development of complications in diabetes. Diabetes vol. 40, pp. 405-412.
5. Bayness, J. Thorpe, S. (1999). Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes vol. 48. Pp. 1-9.
6. Brownlee, M. (2001). Biochemistry and molecular cell biology of diabetic complications. Nature (414): 813-20
7. Caskan, Thorburn, D.R., Pentfold, S.A., Laskowski, .A., Souris, K.C., Thalle-Bonke, V., Fekami, K., Tong, D.C.K, Yab, F.Y.T., Gasser, A., Tan Aly, Harcourt, B., Mubus, A., Pete, J., Brownlee, M., Thorpe, S.R., Bierhaus, A, Copper, M.E., Forbes, J.M., (2009). Rage induced cysteselic Res. Promote mitochondria superoxide generation in diabetes. J. AM. Soc. Nephrol. 20 (4): 725-52.
8. Ceriello, A., and Motz, E. (2004). Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes and cardiovascular disease? The Common Soil hypothesis revisited. Artenoseler Thron Vase Biol (24), 81:816-823.
9. Chong, Z.Z., Li, F., Mavese, K. (2005). Oxidative stress in the brain: Novel cellular targets that govern survival during neurodegenerative disease. Prog. Neurobiol vol. 75, pp. 207-246.
10. De Haan, J.B. (2011). Nrf2 activators as attractive therapeutics for diabetic nephropathy. Diabetes 60 (11): 2683-4
11. Delmastro, M.M., Piganelli, J.D. (2011). Oxidative stress and redox modulation potential in type 1 diabetes. Clin Dev. Immunol. Vol. 1

12. Drews, G., Krippeit-Drews, P., Duffer, M. (2010). Oxidative stress and beta-cell dysfunction pflugers Arch vol. 460, pp. 703-718.
13. Firoozrai, M., Nourbakhsh, M., Razzaghy-Azar, M. (2007). Erythrocyte susceptibility to oxidative stress with type 1 diabetes. Diabetes Res. Clin. Prac Vol. 77, pp. 427-432.
14. Hirsh, I.B. (2005). Intensifying insulin therapy in patients with type 2 diabetes. Am J. med 118 (suppl. 5A) 215-65.
15. Kaneto, H., Katakami, N., Kawamors, D., Miyatsuka, K., Sakamoto, K., Matsuoka, T.A., Matsuhisa, M., Yamasaki, Y. (2007). Involevement of oxidative in pathogenesis of diabetes. Antioxid Redox Signal vol. 9 9, pp. 355-366.
16. Lenzen, S., Drinkgerm, J., Tiedge, M. (1996). Low antioxidant enzyme gene-expression in pancreatic islet compared with various other mouse tissue. Free Radio Biol Med. Vol. 20. Pp. 463-466
17. Maddux, B.A., See, W., Lawrence, J.C. (2001). Protection against oxidative stress-induced insulin resistance in rat 1.6 muscle cells by micromolar concentrations of alpa-lipaic acid. Diabetes (50): 404-10.
18. Miyata, T., Van, Y., Ypersele, S.C., Kurokawa, K., Bayness, J.W. (1999). Alterations in non enzymatic biochemistry in viermia: origin and significance of carboxyl stress in long term cremic stress. Kidney Int. vol. 58, pp. 389-399.
19. Ohkawa, H, Orishi, N., Yagi, K., (1979). Assay for lipid peroxides in animal tissues by thiobabituric acid reaction. Anal Biochem. 95 (2): 351-358.
20. Quagliaro, L. Piconi, Assalone, R. (2003). Intermittent high glucose enhances apoptons related to oxidative stress in human umbilical vein endothehail cells: the role of protein kinase C and NAD(P)H- oxidase activation. Diabetes 52:2745 – 804
21. Rodino-Janeiro, B.K., Gonzolez-Pateiro, M., Alvarez, E. (2010). Glycated albumin, a precursor of advanced glycation en products: A summary of a congress series sponsored by UNESCO MCBN, the German Diabetes Association and the German Diabetes Society. Diabetes Metro Res. Rev. vol. 17, pp. 189-912.
22. Rolo, A.P., Palmeira, C.M. (2006).Diabetes and mitochondrial function: role of hyperglycaemia and oxidative stress. Toxical Appl pharmacol vol. 212 pp. 167-178.
23. Santilli, F.C., Pollone, F., Mezzetti, A. (2004). The role of nitric oxide in the development of diabetic angiopathy. Horm Metab Res. 25: 1439-43.
24. Scheikofer, S., Andrassy, M., Chen, J. (2003). Acute hyperglycemia causes intraceullular formation of CML and activation of ras P42/44 MADK, and nuclear factor? B in PBMCs. Diabetes 52:621-33.
25. Tam, S.M., Stefanovic, N., Tan, G., Wilkinson-Sava, J.L., de Haan, J.B. (2013). Lack of the antioxidant glutathione peroxidase-1 (GP x 1) exercerbate retinopathy of prematurity in mice. Invest opthamol vis Sci. 54(1): 555-62.
26. Tan, Phy, Souric, K.C., Harcourt, B., Thallas-Bonke, V., Penfold, S.A.. Andrikapolous, S., Thomas, M.C., O'Brien, R.C., Bierhaus, A., Copper, M.E., Forbes, J.M., Colaghan, M.T., (2010). Disparate effects on renal and oxidative parameters following RAGE deletion, AGE accumulated inhibition or dietary AGE control in Diabetic nephropathy. Am. J. Physical Renalphysical. 298 (3): F 763-70
27. Telci, A., Catatay, U., Salman, S., Satman, I., Sivas, A. (2000). Oxidative protein damage in early stage type 1 diabetic patients. Diabetes Res. Clin. Prac vol. 50 pp. 213-223.
28. The Diabetes control and complication trial (DCCT) Research Groups (1995). The relationship of glycemic exposure (HbA1c) to the risk of development and progression of retinopathy in the diabetes control and complications trial. Diabetes. 44:968-83

29. Turk, Z. (2010). Glycotoxins, carboxyl stress and relevance to diabetes and its complications. Physio Res vol 59, pp. 147-156.
30. Watson, D., Lowath, A.C. (2009). Oxidative and nitrosative stress in beta-cell apoptosis: Their contribution to beta-cells loss in type 1 diabetes mellitus. Br J. Biomed Sci. Vol. 66 pp. 208-215
31. West, I.C. (2000). Radicals and oxidative stress in diabetes. Diabetic Med. Vol. 17. Pp. 1047-1054.
32. Witko-Sarsat, V., Friedlauder, M., Capeillere-Blandin, C., Nguyen-Khoa, T., Nguyen, .A.T, Zingraff, J., Junger, P., Decamps-Latscha, B. (1996). Advanced oxidation protein products as a novel marker of oxidative stress in uraemia. Kidney Int. Vol. 49, pp. 1304-1313.