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Antioxidant Status and the risk of Diabetic Nephropathy among Patients with Type 2 Diabetic disease: a one-year follow-up study

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Abstract

Hyperglycaemia leads to tissue-damaging due to ill-effect reactions by reactive oxygen species (ROS). Antioxidants therefore evolved to protect against oxidative cell damage. The aim of the study was to assess the antioxidant status and the risk of diabetic nephropathy among patients with type 2 diabetic disease. A prospective study among diabetic patients attending diabetic clinics in Jigawa state, northern Nigeria, approved by the state and hospitals ethical committees was carried out and 130 normo-tensive type 2 diabetic patients and 100 matched control subjects were recruited. Blood pressure (BP), weight and height were measured. Lipid profile, glycaeted haemoglobin (GHbA1c), glucose and malondialdehyde (MDA) were assessed using spectro-photometer, antioxidants using ELISA and albumin creatinine ratio (ACR) immune-turbidimetrically. The data was analyzed statistically using SPSS version 20.0. Out of the 130 patients, 20% were microalbuminuric (M-ALB). Higher and significant MDA in M-ALB throughout the follow ups ($p<0.05$) except at baseline ($p=0.69$). GHbA1c was significantly higher in M-ALB than N-ALB throughout the study. Multiple regression analysis revealed that at baseline, HDL-c has a negative and significant influence on ACR level ($p=0.048$). At

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first follow-up, GHbA1c and MDA were related positively to the development of ACR (p=0.005 and 0.006 respectively). During the second follow up, GHbA1c, MDA, and FBG were related positively while SOD was related negatively (p=0.005, 0.015, 0.021 and 0.019 respectively) to the ACR level. At the third follow up, only MDA was seen to relate to ACR level with (p=0.001). The fourth follow up identified MDA with positive effect while GSH and HDL-c with negative influence on ACR development among the DM patients (p=0.000, 0.046 and 0.026 respectively). Microalbuminuria was seen to be influenced by reduced levels of antioxidants and raised MDA. Careful assessment of antioxidants may detect oxidative damage at an earlier stage. This possibly suggested the benefits of increasing intake of antioxidants through dietary supplements.

Keywords: Hyperglycaemia, Antioxidants, Oxidative stress, Microalbuminuria, Normo-tensive, diabetic nephropathy

Introduction

Diabetes mellitus is a syndrome characterized by hyperglycaemia due to an absolute or relative lack of insulin and or insulin resistance (IDF, 2017). It is a complex, chronic, debilitating disease and is costly in terms of both human suffering and health care expenditure requiring continuous medical care with multi-factorial risk-reduction strategies beyond glycaemic control (IDF, 2013; ADA, 2018).

The hyperglycaemia leads to tissue-damaging that affect a particular subset of cell types like capillary endothelial cells in the retina, mesangial cells in the renal glomerulus and neurons and Schwann cells in peripheral nerves (Rahimi-Madiseh *et al*., 2016). The sustained high intracellular glucose paves way for varied number of ill-effect reactions (Gabbey *et al*., 1966) generating reactive oxygen species (ROS) leading to oxidative stress (Du *et al*., 2000; Nishikawa *et al*., 2000).

As a result of the detrimental effects of ROS, mammalian cells evolved a number of antioxidants to protect against oxidative cell damage. These molecules significantly inhibit oxidation of target molecules through sequestering free transitional metal ions, catalyzing the breakdown of generated oxidants and possibly by scavenging the free radicals to relatively un-reactive radicals (Rizzo and Piston, 2003).

Among the antioxidants, superoxide dismutase (SOD) dismutate the superoxide to form H_2O_2 and later to water by catalase (CAT) or glutathione peroxidase (GPx) which also oxidized the reduced glutathione (GSH) to GSSG and back to GSH by glutathione reductase (GR). Glutathione S-transferase (GST) and GSH as a cofactor is involved in detoxification of organic hydro peroxides and other electrophiles from lipid peroxidation (Hayes *et al*., 2005). In view of the complementary effects of various antioxidants and phase II enzymes in detoxification of ROS and other reactive species, the coordinated actions of a spectrum of cellular antioxidative and phase II defenses are essential for protection against oxidative and electrophilic stress in mammalian tissues, including kidneys (Jakus, 2000).

The recognition of the defective role for ROS in the etiology of various kidney disorders has led to extensive researches on the potential renal protective effects of exogenous antioxidants. Antioxidant vitamins and flavonoids have been used as reno-protective in ischemia-reperfusion injury, transplantation rejection and cyclosporine A-induced nephrotoxicity (Yin *et al*., 2001). However, the studies has yielded inconsistent results, which might be related to the pro-oxidative properties of these compounds (Lee *et al*., 2006; Mahfoudh-Bousssaid *et al*., 2007). Later, over expression of endogenous antioxidative proteins by genetic approaches was

applied to the protection of oxidative renal injury in transgenic mice (Yin *et al.,* 2001). Wider understanding of ROS in biological systems promotes a medical revolution shifting toward disease prevention (Lee *et al.,* 2006). In Nigeria, there is paucity of data on kidney injury assessment in relation to the effect of antioxidant and phase II enzymes among diabetic patients as reno-protective.

Materials and Methods

A consecutive sampling method was adopted where a base line sample for the new and known diabetes mellitus (DM) patients and controls were assessed. A follow-up samples were collected at three month interval for one year from January, 2017 - January, 2018 after screening. Specimen collection of both urine and blood samples were obtained and were fully documented confidentially. Assessments such as blood pressure (BP), waist circumference, weight (W), height (H) and body mass index (BMI) was also observed.

Using aseptic technique, a morning fasting sample of 10 ml venous blood was drawn from each subject and dispensed 5ml each into plane and EDTA containers. The plane samples were centrifuged within 30 minutes of collection using Hettich Universal 32 Centrifuge (Germany). The centrifuged serum and plasma were kept at -20 ^{0}C for two weeks before analysis.
Spot urine specimen was collected on visit. Patients with microalbuminuria were asked to produce two more urine samples at one month interval. Patient and the control subjects were given a clear oral instruction on the urine sample collection and analyzed.

The study considered 68 males and 62 female normo-tensive type 2 diabetic subjects attending endocrine clinic alongside 100 apparently healthy subjects as controls. Fasting venous blood was used to asses biochemical parameters including antioxidants assayed using Enzyme Linked immunosorbent Assay (Melsin medical Co., limited). Enzymatic methods were used for FBG (Barham and Trinder, 1972) and lipid profile for Total Cholesterol (TC) (Trinder, 1999), Triglyceride (TG) (Allain *et al*., 1974), High density lipoprotein (HDL) (McGowan *et al*., 1983) while Low-density Lipoprotein (LDL) was calculated using the formula:

Friedwald equation: $LDL - Chol\ \left(\frac{mmol}{L}\right) = (Total\ Chol.) - (HDL - Chol.) - {}^{TG}/_{22.2}$

Urine was assessed for Albumin creatinine ratio (ACR) in all the diabetic subjects and controls. The relationships between ACR and SBP, DBP, FBG and lipid profile, MDA, GHbA1c and antioxidants status were evaluated. The study was approved by the ethics and review committee of the Jigawa State ministry of health and Specialist Hospital Dutse, Jigawa state, Nigeria, as well as informed consent from all participants using a pre-designed questionnaire.

Statistical Analysis

The data obtained was treated using SPSS version 20.0. Microalbuminuria as (ACR) was compared against normo-albuminuric (N-ALB) and microalbuminuric (M-ALB) groups of the diabetic subjects using student t-test. Other analytes estimated along with the microalbuminuria were assessed using multiple regression analysis to find their influence related to diabetic nephropathy. A p-value ≤ 0.05 was considered statistically significant.

Results

The characteristic of study population was shown in Table 1 with 130 (68 males and 62 females) type 2 diabetic and non-hypertensive patients alongside 100 (74 males and 26 females) as control. Ethnicity was dominated by Hausa (79%), Fulani 15% and Kanuri 7.5%.

Family history of DM, hypertension (HTN) and obesity were indicated in 58%, 32% and 23% respectively.
The results in Table 2 shows that FBG was significantly higher in micro-albuminuric (M-ALB) than normo-albuminuric (N-ALB) (p=0.000) group at follow ups except baseline (p=0.950). GHbA1c was significantly lower in N-ALB compared to M-ALB consistently through the study (p<0.05). MDA was significantly higher throughout the follow ups (P<0.05) except at baseline (p=0.69). SOD was higher and significant in N-ALB than M-ALB patients at first (P=0.003), second and fourth follow ups (p=0.000). The GSH was higher and significant in N-ALB than M-ALB at baseline and second follow ups. GST was higher and significant in N-ALB only at fourth follow up (P=0.05).

Relationship between ACR and GHbA1C, MDA, SOD, GSH, UA, FBG and HDL-CHOL is shown on Table 3. At baseline, HDL-c has a negative and significant influence on ACR level (p=0.048). At first follow-up, GHbA1c and MDA were related positively to the development of ACR (p=0.005 and 0.006 respectively). During the second follow up, GHbA1c, MDA, and FBG were related positively while SOD was related negatively (p=0.005, 0.015, 0.021 and 0.019 respectively) to the ACR level. At the third follow up, only MDA was seen to relate to ACR level with (P=0.001). The fourth follow up identified MDA with positive effect while GSH and HDL-c with negative influence on ACR development among the DM patients (P=0.000, 0.046 and 0.026 respectively).

Table 1: Socio-Demographic Characteristics of the Study Participants

Variables	Control n (%) (n=100)	Patients n (%) (n=130)
Gender		
Male	74 (74)	68 (52.3)
Females	26 (26)	62 (47.7)
Ethnicity		
Hausa	91 (91)	103 (79.2)
Fulani	7 (7)	20 (15.4)
Kanuri	2 (2)	7 (5.4)
FHDM		
Yes	52 (52)	75 (57.7)
No	48 (48)	55 (42.3)
FHHTH		
Yes	26 (26)	41 (31.5)
No	74 (74)	89 (68.5)
F HO		
Yes	11 (11)	30 (23.1)
No	89 (89)	100 (76.9)
Education Status		
Non-formal	38 (38)	50 (38.4)
Primary	4 (4)	6 (4.6)
Secondary	15(15)	21 (16.2)
Tertiary	43(43)	53 (40.8)
FGI		
High	93 (93.00)	104 (80)
Low	7(7.00)	26 (20)

Key: n=population, size **FGI=**Food glycaemic index, FHO=Family history of Obesity, FHHTN=Family History of Hypertension, FHDM=Family History of Diabetes

Table 2: Mean FBG, Oxidative Stress Marker and Antioxidants among Normoalbuminuric and Microalbuminuric Diabetic Patients at Baseline and Follow-Ups

Parameters	Category n=100/25	Baseline Mean±SD	p- value	3 months Mean±SD	p-value	6 months Mean±SD	p-value	9months Mean±SD	p-value	12months Mean±SD	p-value
FBG (mmol/L)	N-ALB	6.27±2.65	0.950	6.90±2.37	0.000*	7.43±1.86	0.000*	8.50±1.74	0.000*	9.44±2.15	0.001*
	M-ALB	6.23±3.54		11.50±1.90		11.47±1.48		10.81±1.36		11.20±2.18	
MDA (mmol/L)	N-ALB	13.94±2.85	0.690	14.02±2.78	0.001*	13.46±2.56	0.000*	13.34±2.33	0.000*	12.43±2.08	0.000*
	M-ALB	14.20±3.31		16.03±2.32		16.61±2.46		16.33±1.74		14.82±2.61	
SOD (ng/ml)	N-ALB	3.70±0.65	0.160	3.82±0.77	0.003*	3.88±0.78	0.000*	4.14±3.79	0.321	3.73±0.55	0.000*
	M-ALB	3.89±0.51		3.28±0.81		3.03±0.51		3.31±0.41		3.26±0.44	
GSH (ng/L)	N-ALB	32.89±3.19	0.050*	32.11±3.30	0.198	31.77±3.22	0.005*	30.51±3.07	0.260	30.67±3.35	0.066
	M-ALB	34.24±2.45		30.86±6.83		29.58±4.35		29.71±2.26		29.24±2.84	
GST (ng/ml)	N-ALB	31.02±5.47	0.960	34.70±43.86	0.557	29.90±5.35	0.173	30.06±4.20	0.233	34.08±40.72	0.050*
	M-ALB	30.96±5.83		29.50±5.88		28.30±5.24		28.90±3.28		28.61±4.00	
GHbA1C (%)	N-ALB	7.60±1.56	0.040*	7.78±1.43	0.000*	8.18±0.97	0.000*	8.53±1.34	0.000*	9.33±1.35	0.009*
	M-ALB	11.55±19.73		9.59±2.77		10.99±1.96		9.68±1.25		10.16±1.29	

MDA-malondialdehyde; SOD-superoxide-dismutase; GSH-glutathione; GST-glutathione-s-transferase; UA-uric acid; FBG-fasting blood glucose; GHbA1c-glycaeted haemoglobin A1c

Table 3 Influence of MDA, GHbA1c, SOD, GSH, GST, UA and HDL-C on ACR in Multiple Regression Analysis

Dependant variable	Independent variables	Base line		3-month		6-month		9-month		12-month	
				β	p-value	β	p-value	β	p-value	β	p-value
ACR	GHbA1C	0.107	0.232	**0.227**	**0.005***	**0.256**	**0.005***	0.064	0.459	0.094	0.167
	MDA	0.105	0.256	**0.205**	**0.006***	**0.200**	**0.015***	**0.315**	**0.001***	**0.257***	**0.000***
	SOD	-0.000	0.997	-0.150	0.059	**-0.185**	**0.021***	0.013	0.865	-0.148	0.062
	GSH	-0.159	0.067	-0.028	0.708	-0.022	0.769	-0.099	0.262	**-0.167**	**0.046***
	GST	-0.041	0.630	-0.012	0.870	-0.035	0.640	-0.080	0.368	-0.011	0.869
	UA	0.039	0.656	**0.159**	**0.038***	0.086	0.229	0.088	0.268	0.022	0.745
	FBG	0.066	0.949	0.090	0.390	**0.291**	**0.019***	**0.060**	**0.560***	0.046	0.699
	HDL-C	**-0.753**	**0.048***	0.341	0.161	-0.579	0.118	0.052	0.832	**-0.688**	**0.026***

MDA-malondialdehyde; SOD-superoxide-dismutase; GSH-glutathione; GST-glutathione-s-transferase; FBG-fasting blood glucose; UA-uric acid; HDL-C high density lipoprotein-cholesterol; GHbA1c- glycaeted haemoglobin; *-significant at p-value ≤0.05

Discussion

Diabetes mellitus (DM) is a common metabolic condition worldwide. Among its striking complications is diabetic nephropathy (DN) which is the primary cause of end-stage renal

disease aetiology. The metabolic dysregulation associated with DM commonly leads to oxidative stress that affects various tissues causing many complications including kidney injury.
In this study, an elevated GHbA1c increases the risk of microalbuminuria over time as there was a simultaneous linear increase in both GHbA1c status and microalbuminuria as the control worsens. Also found that patients with poor glycaemic control have higher GHbA1c when compared with control group. This signified the clinical usefulness of GHbA1c assay in evaluating glycaemic control in this environment. Higher and significant differences were also observed in M-ALB than N-ALB patients. The strong influence on both MDA and ACR as shown in this study further confirmed its utility in prognosis of the disease. In contrast, most of the available studies from sub-Saharan African (Lutale *et al.,* 2007) fail to demonstrate any significant relationship between the level of glycaemic control and microalbuminuria. Poor glycaemic control is a well defined contributor to the development and progression of microalbuminuria among Type 2 patients (DCCTs, 1993; UKPDS, 1998; ADA, 2018).

In this study, it was observed that TC, LDL-C and TC/HDL-C levels were found to be significantly higher with corresponding low HDL-C in diabetic patients than control group. Similar findings were reported by Murwan *et al*. (2016) in Sudan and Wamique *et al.* (2016) in India. Low HDL-C level could be related to decreased LCAT activity or due to increased LDL and VLDL as there is a reciprocal relationship between HDL-C and LDL (Kaviarasan *et al*., 2005) leading to decreased HDL-C protection (Gowri *et al*., 1999). According to Sabahelkhier *et al*. (2016) and Farah *et al.* (2013) there is significant elevation in VLDL-C and corresponding decrease in HDL-C among type 2 diabetic patients when compared with controls. This study demonstrated similar results in addition to TC and LDL-C which were significantly elevated. The reasons behind increased TC and LDL-C are increasing in the incidence of the obesity, sedentary life style or physical inactivity, diet and risk factors like ethnicity. Contrary to the reports by Murwan *et al*. (2016) and Sabahelkhier *et al*. (2016), this study observed a higher but insignificant TG in patient than control group. Decreased triglyceride level was significantly associated with African ancestry compared to Europeans (Deo *et al*., 2009). The explanation for interethnic lipid variability may be potentially linked to the activity of lipoprotein lipase (LPL) that hydrolyses TG having significantly greater LPL activity in African ancestors than European ancestors (Deo *et al*., 2009; Bentley and Rotimi, 2012; Muazu *et al*., 2017). Other possible significant predictors of TG after LPL adjustment could be sex, fat mass and visceral adipose tissue (Deo *et al*., 2009; Despre's *et al*., 2000).

Significant decrease in antioxidant activity was observed among the diabetic patients compared to healthy controls. This could relate to the limiting antioxidant capacity for an increased MDA level observed in addition to other unmeasured oxidative stress biomarkers in this study as supported by Sharma *et al*. (2016). This could enhance the progression to chronic diabetic complications at much faster rate commonly observed among patients with poor control (ADA, 2018). It can therefore be suggested that insufficient detoxification effect by antioxidants may lead to an imbalance system of oxidants and antioxidants.

In this study, SOD was observed to influence MDA and ACR development in a negative passion as reported by Hisalkar *et al*. (2012). This could be explained by the deleterious effect of DM resulting to huge concentration and greater oxidative stress or as a result of feedback inhibition on SOD activities (Al-shebly and Mansour, 2012).

The GST activity in this study was significantly lower with increased ROS when compared to control group as similarly reported by Mohammed *et al.* (2015). Similar report was given by

Varghese *et al*. (2012) on patients with periodonitis. This could be due to alteration of GST activity due to hyperglycaemia, enzyme gene expression or a post translational enzyme glycaetion or both (Wolff *et al*., 1991; Mohammed *et al*., 2015). Hyperglycaemia, lipoxidation and glyco-oxidation products results to HbA1c and AGE both implicated in oxidative stress leading to endothelial dysfunction in absence of compensatory mechanisms (Giera *et al*., 2012) typical to our findings in patients with significantly higher FBG, GHbA1c and lipid profile.
The influence of MDA alongside other unmeasured stress markers may likely be detrimental to renal function with increased duration of the illness. This is evidently seen as the study shows a progressive increase in concentration of MDA that keeps on throughout the period of one year follow ups. Studies have shown that hyperglycemia even in non-diabetic rats can increase muscle protein carbonyl content and the concentrations of MDA and 4-hydroxynonenal as indicators of lipid peroxidation and ROS (Nasri *et al*., 2013; Bahmani *et al*., 2014; Rahimi-Madeshi *et al*., 2016). MDA was elevated in patients with M-ALB and increases significantly in parallel with ACR levels through the study period.

The findings therefore unraveled some steps in treatment and prevention of microalbuminuria and deleterious effect of hyperglycaemia through effective use of antioxidants. Evidences may also be drawn to close-up some of the gaps to support the burden of kidney disease in African people with DM. It will therefore be of meaningful approach as a strategic therapy in combating hyperglycaemia and oxidative stress economically with little or no clinical toxic effect to successfully treat and/or prevent hyperglycaemia and diabetic nephropathy.

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