Original Research Article

CORRELATION BETWEEN OXIDATIVE STRESS MARKERS AND ATHEROGENIC INDICES IN TYPE 2 DIABETES MELLITUS

ABSTRACT

Worldwide, approximately 200 million individuals are currently suffering of type 2 diabetes mellitus (DM). Diabetes mellitus is associated with hyperglycemia, which induces oxidative stress that is responsible for the various complications associated with the disease. This study was designed to know the relationship between oxidative stress and atherogenic indices of plasma in type 2 diabetic and non-diabetic subjects. A total number of eighty (80) subjects comprising 58 diabetic subjects with mean age (62.91±10.57) years and 22 non-diabetic subjects with mean age (55.27±16.62) years were studied. Estimation of enzymatic and non-enzymatic oxidative stress markers (which include MDA, SOD, GPx, CAT, Uric acid and Albumin) and atherogenic indices (TCHOL, TG, HDL, LDL) were done respectively using standard spectrophotometric techniques. The plasma mean of SOD, GPx, CAT and albumin were significantly lower in diabetic subjects compared with control group. However, TChol, HDL, MDA and uric acid were significantly higher in diabetic subjects compared with controls. The findings of this study show significant differences in dyslipidemia, lipid peroxidation and increasing of oxidative stress markers from naïve type 2 diabetic subjects through controls. Thus, early diagnosis and management of this condition is necessary in order to incorporate antioxidant supplement as a supportive therapy for adequate glycaemic control.

Keywords: Diabetes mellitus, Oxidative stress, antioxodant, CVD, atherogenic indices

1.0 INTRODUCTION

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic diseases characterized by chronic hyperglycaemia over a prolonged period. Diabetes is either due to the pancreas inability to produce adequate insulin or insulin resistant to the cells of the body [1]. As of 2014, estimated 387 million diabetes cases have been reported worldwide [2] with type 2 DM making up about 90% of the case [3].

This represents 8.3% of the adult population with equal rates in both women and men [4]. From 2012 to 2014, diabetes is estimated to have resulted in 1.5 to 4.9 million deaths each year and the number of individuals with diabetes are expected to rise to 592 million by 2035 [5]. Diabetes has been reported to at least double individuals' risk of death [6].

There are three main types of diabetes mellitus as reported by Picot *et al.* [7]: Type 1 DM, type 2 DM and gestational diabetes. Inability of the pancreas to produce enough insulin is the main cause of type 1 DM and this type was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes" [4]. Type 2 DM begins with insulin resistance; a condition in which cells fail to respond to insulin properly [4]. As the disease progresses, a lack of insulin may also develop [8]. This form was previously referred to as "non-insulin-dependent diabetes mellitus" (NIDDM) or "adult-onset diabetes". The primary cause is excessive body weight and inadequate exercise [4]. Gestational diabetes is the third main type and occurs when pregnant women without a previous history of diabetes develop hyperglycaemic condition [4]. Type 2 diabetes is typically a chronic disease associated with a ten-year-shorter life expectancy. Long-term complications from this condition includes heart disease, strokes, diabetic retinopathy, kidney failure, and poor blood flow in the limbs leading to amputations [1].

Free radicals are atoms or group of atoms with an unpaired number of electron(s) in their outer most shell and can be possibly formed when oxygen interacts with certain biomolecules [9]. Once formed, these highly reactive species can start a chain reaction. Their chief danger comes from the damage they can do when they react with important cellular component such as DNA, or the cell membrane [9]. Cells might function poorly or die if this eventually occurs and could not be arrested on time. To prevent free radical effect(s), the body has a defense mechanism system of antioxidant [10]. An antioxidant is a molecule that inhibits the oxidation of other molecules, while oxidation is a chemical reaction that can produce free radicals, leading to chain reaction that may damage cells. Thus antioxidant such as thiols or ascorbic acid terminates this chain reaction [11]. To balance the oxidative state, plant and animal maintain complex system of overlapping antioxidants, such as glutathione and enzymes (such as catalase) produced internally or Vitamin C, Vitamin A, and Vitamin E obtained by ingestion [12]. Antioxidants are widely used in dietary supplements and have been investigated to be highly effective for the prevention of diseases such as cancer and coronary heart diseases [13].

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells could possibly cause toxic effects through the production of peroxides and free radicals that damage body's biomolecules, including proteins, lipids, and DNA [14]. Oxidative stress from oxidative metabolism has been reported to cause base damage, as well as break strand in DNA [15]. In humans, oxidative stress is thought to be involved in the development of atherosclerosis and had been sited to be of etiological importance in cardiovascular diseases [16], which could be related to diet and also metabolic disorders with abnormal lipid metabolism [17]. In either of the case it results to atherosclerotic endothelial dysfunction from arterial diseases and this has been reported to be responsible for about 30% of deaths worldwide [16]. Diabetes mellitus is characterized with hyperglycemia, which may induce oxidative stress that is responsible for the various complications associated with the disease [18], which affects the heart, the nerves and the retina resulting into heart disorders [19]. The characteristics of diabetic mellitus such as hyperglycemia, dyslipidemia, inflammation and oxidative stress affect the vascular wall and thus accelerate atherosclerosis and its clinical complications [10]. Atheroslerotic disorder of the coronary arteries usually result in partial or complete occlusion of vascular lumen and this is of pathologic significance in determining the morbidity and mortality pattern of ischemic heart disease (IHD) [10]. Coronary artery disease (CAD) is initially symptomless with normal basic activities but as the disease progresses, the degree of lumen narrowing is sufficiently great and this limits increase in blood flow during exercise and thus producing symptoms of angina pectoris which can lead to heart attack [20].

Oxidative stress is associated with increased production of oxidizing species or a significant decrease in the effectiveness of antioxidant defenses, such as glutathione [21]. The effects of oxidative stress depend upon the size of these changes, with a cell being able to overcome small perturbations and regain its original state. However, more severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stress may cause necrosis [22]. Worldwide, approximately 200 million individuals are currently suffering of type 2 diabetes mellitus (DM) [2]. Some studies have shown that Type 2 DM subjects generally carry a number of risk factors for coronary vascular disease (CVD),

which is found to be characterized with hyperglycemia, abnormal lipid profiles pattern and alterations in inflammatory mediators [20]. Thus, diabetes mellitus associated with cardiovascular diseases tends to be one of the highest causes of death worldwide. This study therefore aimed to know the relationship between oxidative stress biomarkers and atherogenic indices of plasma in type 2 diabetes mellitus, which might contribute to the incidence of CVD in this condition if it is not ameliorated on time.

2.0 MATERIALS AND METHODS

2.1 Study population

This study was conducted at Federal Medical Centre, Owo, Ondo State. Owo is a town in Ondo State situated at south-western Nigeria, with latitude 710'59.998''N and longitude 534'59.988''E at an average altitude of 348 meters. It is at the southern edge of the Yoruba hills, and at the intersections of roads from Akure and Benin City. The community has a population of 276, 593 according to national population in the year 2006 census [23].

2.2 Study design

This is a case-control study and it was conducted at Federal Medical Centre, (FMC) Owo, which serve as tertiary health institutions in Ondo State. The research was conducted between January to July, 2016. A total number of eighty (80) subjects which include fifty eight (58) type 2 diabetes mellitus subjects (both males and females) aged between 30 – 80 years, which were sub-divided into diabetic mellitus subjects under treatment, DMUT and naïve diabetic subjects (which are newly diagnosed type 2 diabetes mellitus) attending diabetic clinic at Federal Medical Centre, Owo were randomly selected for this study. Type 2 diabetes mellitus subjects in this study were diagnosed according to guideline of WHO [24]. Their medical history and personal data was obtained via short structured questionnaire after due approval from the ethical committee of the hospital. Twenty two (22) age and sex matched apparently healthy controls with no history of diabetes mellitus were enrolled into this study. Informed consent was thus obtained from all the participants.

2.3 Ethical clearance and consent

Subjects participating in this study were fully briefed on the research protocols in the clinic after which they were required to sign a written consent. After that, a pre-designed structural questionnaire was utilized to collect bio-data, and socio-demographic characteristics of the patients. Approval for this study was obtained from the Federal Medical Centre, Owo and Ethical Clearance (FMC/OW/380/VOL.XXIX/197) was issued by Ethical Committee Federal Medical Centre, Owo.

2.4 Collection and Storage of Samples

Blood samples were obtained from each subject by applying a tourniquet around the arm above elbow. The ante-cubital forsa was disinfected with a 70% alcohol soaked swab. Six milliliters (6ml) of venous blood was collected from each subject using aseptic procedure after 12 hours fast. Four milliliters (4ml) of venous blood was dispensed into 5 ml sterile vacutainer bottle containing lithium heparin anticoagulant and gently mixed by inverting the container severally for the determination of lipids profile and oxidative stress markers. The remaining (2mls) of the venous blood was dispensed into 3mls vacutainer bottle containing fluoride oxalate anticoagulant which was also mixed gently by inverting the container several times for the determination of plasma glucose. Plasma was separated from the blood by centrifugation for 5 minutes at 4000rpm, into plain bottles and stored at -20℃ until time of analysis.

2.5 Analytical Methods

Height (m) was taken using a Stadiometer while body weight (kg) was taken using a body weight weighing scale with the subject wearing light clothing and without shoes. Body mass Index (BMI) was calculated as the ratio of weight (kg) to the square of height (m^2). Blood pressure and pulse rate were taken simultaneously using a sphygmomanometer. Blood levels of fasting blood sugar and lipids profile were determined using standard spectrophotometric method [25] and standard methods were employed for the determination of SOD, CAT and GPx plasma activities [26, 27] and plasma levels of MDA, Uric acid and albumin [28, 29, 30].

2.6 Statistical analysis of data

A statistical package for social sciences (SPSS) 17.0 was used for the analysis of the data appropriately. All values were expressed as Mean ± Standard deviation (SD). Analysis of variance (ANOVA) was used to determine significant differences among groups while Spearman correlation was used to test the

association between variables. The level of significance was taken at 95% confidence interval and P value less than 0.05 was considered significant.

3.0 RESULTS AND DISCUSSION

3.1 Results

A total number of eighty (80) subjects comprising 58 diabetic subjects with mean age (62.91±10.57) years and 22 non-diabetic subjects (control) with mean age (55.27±16.62) years were studied. Twenty three (23) out of the diabetic subjects were naïve (i.e. not yet placed on diabetic drugs) while the remaining 35 were already undergoing treatment.

Table 1 shows the age and sex distribution of all participants. Participants were aged between 30 and 80 years. There were 34 females and 24 males, and 13 females and 9 males in diabetic and non-diabetic groups respectively. Thus, females constituted 58.75% while males constituted 41.25% in overall.

Table 2 shows the anthropometric indices and biochemical parameters in naïve diabetic subjects (naïve DM), diabetic subjects under treatment (DMUT) and controls using One way analysis of variance (ANOVA). The mean BMI, Pulse, SBP, DBP, FBS, TChl, TAG, HDL, MDA and Uric acid were significantly higher in naïve DM than controls while the mean plasma of albumin and plasma activities of SOD, GPx and CAT were significantly lower. Also, the mean BMI, Pulse, SBP, DBP, FBS and HDL were significantly higher, whereas plasma activities of SOD, GPx and CAT were significantly lower in DMUT than controls. However, the mean Pulse, SBP, DBP and FBS were significantly lower, whereas mean plasma of albumin was significantly higher in DMUT compared with naïve DM.

Table 3 indicates correlation of plasma levels of enzymatic antioxidant biomarkers with atherogenic indices and other parameters in diabetic subjects. CAT had positive correlation with FBS, TChol, TAG and LDL, but inverse correlation with HDL. Also, SOD showed statistically negative correlation with TChol, TAG, HDL and LDL, while GPx only had positive correlation with HDL, TChol:HDL and LDL:HDL. Finally, table 4 shows plasma levels of MDA had significant positive correlation with FBS, TChl and LDL. Uric acid showed statistical positive significant correlation with blood pressure (SBP and DBP), while albumin only had significant inverse correlation with pulse.

Table 1: Age and Sex distribution of the Subject population in percentage (%)

Age group (Years)	Diabetic Subjects		Non-diabetic subjects		Total
	Male	Female	Male	Female	
31-40	-	1 (1.25)	4 (5)	3 (2.75)	8 (10)
41-50	8 (10)	2 (2.5)	-	3 (3.75)	13 (16.25)
51-60	2 (2.5)	8 (10)	1 (1.25)	2 (2.5)	13 (16.25)
61-70	10 (12.5)	15 (18.75)	1 (1.25)	3 (2.75)	29 (36.25)
71-80	4 (5)	8 (10)	3 (3.75)	2 (2.5)	17 (21.25)
Total	24 (30)	34 (42.5)	9 (11.25)	13 (16.25)	80 (100)

Table 2: Anthropometric indices and biochemical parameters in naïve diabetic subjects, diabetic subjects under treatment (DMUT) and controls

Parameters	Naïve DM (n=23)	DMUT (n=35)	Control (n=22)
BMI (Kg/m^2)	30.44±6.28[a]	27.94±8.42	24.88±5.11
Pulse (b/m)	78.04±4.65[a]	72.51±4.13[a, b]	69.09±3.04
SBP (mmHg)	135.00±12.61[a]	125.31±12.38[a, b]	115.73±8.69
DBP (mmHg)	85.43±7.22[a]	81.57±7.65[a, b]	75.64±5.38
FBS (mmol/l)	13.50±4.95[a]	8.47±3.45[a, b]	4.57±0.61
TChl (mmol/l)	5.38±1.37[a]	4.82±1.31	4.26±1.01
TAG (mmol/l)	2.21±0.86[a]	1.53±0.62	1.46±0.74
HDL (mmol/l)	1.44±0.38[a]	1.40±0.53[a]	1.04±0.27
LDL (mmol/l)	2.93±0.93	2.72±0.90	2.56±0.62
TChl:HDL	3.95±1.64	3.70±1.12	4.20±0.74
LDL:HDL	2.21±1.29	2.18±0.94	2.57±0.68
SOD (U/ml)	2.12±0.47[a]	1.96±0.81[a]	3.19±1.39
MDA (µmol/l)	3.44±1.07[a]	3.12±1.66	2.51±0.96

GPx (U/ml)	2.10±0.68 [a]	1.99±0.79 [a]	2.90±0.90
CAT (U/L)	19.76±5.71 [a]	21.23±7.11 [a]	27.91±6.87
Uric Acid (mmol/l)	437.30±155.11 [a]	363.41±182.41	287.99±125.75
Albumin (mg/dl)	33.56±5.07 [a]	38.35±5.07 [b]	38.39±4.10

[a] = significantly different from controls, [b] = significantly different from Naïve DM,

Key: BMI= body mass index, SBP= systolic blood pressure, DBP= diastolic blood pressure, FBS= fasting blood sugar, TChl = Total cholesterol, TAG = triglycerides, HDL = High density lipoprotein, LDL = Low density lipoprotein, SOD = Superoxide dismutase, MDA= Malondialdehyde, GP_X = Glutathione peroxidase, CAT= Catalase

Table 3: Correlation of plasma levels of enzymatic antioxidant biomarkers with atherogenic indices and other parameters in diabetic subjects

	SOD		GPx		CAT	
	r-value	**p-value**	**r-value**	**p-value**	**r-value**	**p-value**
BMI (Kg/m^2)	-0.151	0.259	0.040	0.765	-0.096	0.474
Pulse (b/m)	0.130	0.332	0.108	0.419	0.015	0.910
SBP (mmHg)	-0.033	0.807	-0.063	0.638	- 0.159	0.234
DBP (mmHg)	-0.112	0.404	-0.127	0.342	-0.253	0.056
FBS (mmol/l)	-0.064	0.635	-0.250	0.059	-0.373	0.004*
TChl (mmol/l)	-0.474	0.000*	-0.235	0.076	-0.447	0.000*
TAG (mmol/l)	-0.279	0.034*	- 0.104	0.439	-0.387	0.003*
HDL (mmol/l)	-0.308	0.019*	0.286	0.029*	0.303	0.021*
LDL (mmol/l)	-0.418	0.001*	0.070	0.603	-0.293	0.026*
TChl:HDL	-0.010	0.938	0.297	0.024*	0.096	0.474
LDL:HDL	0.001	0.994	0.287	0.029*	0.132	0.324

* Correlation is significant at the 0.05 level (2-tailed)

Table 4: Correlation of plasma levels of non-enzymatic biomarkers of oxidative stress with atherogenic indices and other parameters in diabetic subjects

	MDA		Uric Acid		Albumin	
	r-value	p-value	r-value	p-value	r-value	p-value
BMI (Kg/m^2)	0.230	0.083	0.025	0.852	-0.155	0.244
Pulse (b/m)	0.121	0.366	0.184	0.166	-0.307	0.019*
SBP (mmHg)	0.101	0.450	0.312	0.017*	-0.121	0.366
DBP (mmHg)	0.231	0.081	0.291	0.027*	0.096	0.472
FBS (mmol/l)	0.382	0.003*	0.251	0.057	-0.321	0.014
TChl (mmol/l)	0.512	0.000*	-0.031	0.818	-0.036	0.790
TAG (mmol/l)	-0.336	0.010*	- 0.052	0.697	-0.192	0.149
HDL (mmol/l)	0.168	0.206	-0.073	0.587	-0.113	0.398
LDL (mmol/l)	0.460	0.000*	- 0.048	0.718	0.010	0.938
TChl:HDL	0.147	0.272	-0.057	0.674	0.034	0.798
LDL:HDL	0.113	0.400	- 0.072	0.591	0.068	0.613

* Correlation is significant at the 0.05 level (2-tailed)

3.2 Discussion

Diabetes mellitus is associated with hyperglycemia which induces oxidative stress that is responsible for the various complications associated with the disease [18], which affects the heart, the nerves and the retina resulting into heart disorders [19]. The characteristics of diabetic mellitus such as hyperglycemia, dyslipidemia, inflammation, and oxidative stress affect the vascular wall and thus accelerate atherosclerosis and its clinical complications [10].

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Oxidative stress depicts the existence of products called free radicals and reactive oxygen species (ROS) which are formed under normal physiological conditions but become deleterious when they are unable to be quenched by the antioxidant systems [31]. There are convincing experimental and clinical evidences that the generation of reactive oxygen species is increased in both types of diabetes

and that the onset of diabetes is closely associated with oxidative stress [32]. Free radicals are formed disproportionately in diabetes by glucose autoxidation, polyol pathway and non-enzymatic glycation of proteins [33]. Abnormally high levels of free radicals and simultaneous decline of antioxidant defense systems can lead to the damage of cellular organelles and enzymes, increase lipid peroxidation and development of complications of diabetes mellitus [34].

From the results obtained in this study, it is evident that the diabetic patients had much higher glucose and lipids levels (TChol and TAG) when compared with non-diabetic subjects. Increased of these indices in this work is consistent with Whiting et al. [35] who reported that chronic hyperglycemia could influence the generation of free radicals, which might eventually lead to increased lipid peroxidation and depletion of antioxidants. Significant lipid peroxidation, higher levels of lipids and lipid risk factors (such as increase in BMI, SBP & DBP) in diabetic subjects in this study are indicators for atherogenic changes [36]. The products of lipid peroxidation are harmful to most cells in the body and are associated with a variety of diseases, such as atherosclerosis and brain damage [37].

The major finding of this study was that antioxidant levels, both enzymatic and non-enzymatic, were either significantly reduced or increased in diabetic subjects. Significantly decrease in albumin levels and elevated levels of uric acid in the diabetic subjects when compared with the corresponding control groups are reflective of the acute phase response. Acute-phase reactants are plasma proteins that alter in concentration sequel to an inflammatory stimulus [38]. Thus, decrease in plasma levels of albumin may be used as a marker of negative acute phase proteins in type 2 diabetic subjects.

Significantly increased mean levels of plasma Uric acid in naïve diabetic cases when compared to controls is associated with the cause of type 2 diabetes, independent of obesity, dyslipidemia and high blood pressure as reported by Dhengan et al. [39]. In humans, uric acid is the main plasma antioxidant followed by vitamin C and thus, it stabilizes vitamin C in plasma and protects it from oxidation [40, 41]. Besides that hyperuricaemia was presumed to be consequence of insulin resistant [39], Uric acid in the blood had also been documented to scavenge superoxide radicals, hydroxyl radicals, singlet oxygen and

could chelate transition metals [42]. Thus increase in plasma levels of Uric acid in cases compared to controls might be a compensatory mechanism to mump up free radicals generated in diabetic condition.

This study shows a significant increase in plasma MDA levels in type 2 diabetics when compared to controls indicating increase lipid peroxidation. Malondialdehyde (MDA) is a product of lipid peroxidation and provides a means of assessing the extent of lipid peroxidation. Our data show plasma levels of MDA had significant positive correlation with FBS and TChol. This finding is in agreement with previous report by Suchitra et al. [36] who also reported significant positive correlation of MDA with FBS and TChol in diabetic subjects. This correlation analysis also suggests that hyperglycemia per se is greatly involved in oxidative stress resulting in increased lipid peroxidation.

The significant reduction in activity of serum antioxidant enzymes such as SOD, CAT and GPx was recorded in this work among diabetic subjects when compared to controls. This observation is consistent with most invivo and invitro studies which demonstrated that the levels of antioxidant enzymes are altered in chronic conditions [43]. Catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS) [44]. Superoxide dismutases are important antioxidant defense systems in nearly all cells exposed to oxygen, they are proteins co-factored with copper and zinc, or manganese, iron, or nickel, while GPx is a selenium dependent enzyme with peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water [45]. Oxidative stress results when there is increased production of free radicals or decreased activity of counter-actors, antioxidants or both in a combination [36]. These observations provide evidence why there is increased in the oxidative stress among type 2 diabetes.

4.0 Conclusion and recommendations

The findings of this study show significant differences in dyslipidemia, lipid peroxidation and increasing of oxidative stress markers from naïve type 2 diabetic subjects through controls. Thus, early diagnosis and management of this condition is necessary in order to incorporate antioxidant supplement as a supportive

246 therapy for adequate glycaemic control. This would go far in preventing development of oxidative stress-
247 associated diabetic complications.

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