Oxidative Stress Correlates with Complications Among Diabetic Patients Attending a Diabetic Clinic in Muhimbili National Hospital, Dar es Salaam, Tanzania

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Abstract In diabetes, persistent hyperglycemia results in increased production of free radicals especially oxygen free radicals, which can cause cell destruction and tissue injury resulting in cell dysfunction. With the premise that oxidative stress is a major cause of diabetic complications, we conducted a controlled laboratory based investigation on level of lipid peroxide levels in the serum of Type 1 and Type 2 diabetic patients attending Muhimbili National Hospital. From our clinical data it was observed that majority of the patients had higher waist to hip ratio and body mass index, which suggests that the patients were either overweight or obese. The enrolled diabetic patients had higher lipid peroxide levels than controls and also Type 2 patients had higher lipid peroxide levels than Type 1 patients. Moreover, patients with known complications had higher lipid peroxide levels than patients without complications. The lipid peroxide levels in the diabetic patients were significantly different from that of the control subjects enrolled in the study. A majority of the diabetic patients had a poorly controlled blood sugar. Our finding hints that despite the fact that diabetic patients in our clinic are on follow up, they are at a risk of developing coronary heart diseases, neuropathy and other secondary diabetic complications.

Keywords Oxidative stress · Lipid peroxidation · Diabetes · Diabetic complications

Introduction

Diabetes is a chronic metabolic disorder characterized by high levels of glucose in blood termed as hyperglycemia. This is due to decreased uptake of glucose from blood in the absence or shortage of insulin or due to insulin resistance, especially by insulin dependent tissues like skeletal muscle and adipose tissue. Chronic hyperglycemia results in glycation of a number of proteins. Many studies showed that free radicals are formed during diabetes by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins [1–3]. This process results in acute endothelial dysfunction and activation of inflammation in blood vessels of patients with diabetes, and these factors contribute to the development of complications of diabetes. Oxidative stress plays an important role in the pathogenesis and the complications of diabetes. Oxidative stress occurs when free radical production exceeds the body's ability to neutralize them using the available endogenous biological antioxidant systems, which include: enzymes like superoxide dismutase, catalase, glutathione reductase and non enzymatic antioxidant molecules like glutathione, vitamins E, and C. Oxidative stress functions on both sides, meaning that it helps the progression and the development of diabetes and its complications [4]. A very good description of diabetes and its cause of complications was published by Brownlee in a review [5].

We conducted a small study in our university diabetic clinic to reveal whether or not our diabetic patients were in oxidative stress by way of measuring lipid peroxide levels

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in serum of patients and did a correlation analysis between the relationships of oxidative stress with diabetic complications. Such a study is the first of its kind in Tanzania and would give better information to physicians, patients, the health authorities and the science community regarding the status of patients and measures that have to be taken to treat the disease. Diabetes can lead to derangements in lipid profiles followed by lipid peroxidation, which precipitates to cellular injury and atherosclerosis as major incidences of complications [6, 7]. It is important to continue to explore the relationship between free radicals, diabetes, and the resulting complications, and to elucidate the mechanisms by which increased oxidative stress accelerates the development of diabetic complications, in an effort to expand treatment options. Cardiovascular diseases, characterized by endothelial dysfunction and accelerated atherosclerosis, are the leading cause of morbidity and mortality associated with diabetes. There is growing evidence that excess generation of highly reactive free radicals, largely due to hyperglycemia, causes oxidative stress, which further exacerbates the development and progression of diabetes and its complications [8, 9].

Materials and Methods

This was a cross-sectional case control study made on eighty diabetic patients (12 Type 1 and 68 Type 2) and thirty control subjects. Out of the 80 patients 57 (71.2 %) were female and 23 (28.8 %) were male. The diabetic patients were randomly selected from among the Thursday clinic attendants and the control subjects were randomly selected from students, administrative workers and other volunteers who had no history of diabetics, not pregnant, and not taking any medication for dyslipidemia. Ethical clearance was obtained from the Muhimbili University of Health and Allied Sciences (MUHAS) Institutional Review Board (IRB). Body mass index (BMI), waist to hip ratio (WHR), Blood pressure (BP), and the patient legs were inspected for deformity, foot ulcer and skin color change followed by a 10 g monofilament test. At the clinic each patient's Fasting blood glucose (FBG) was determined using Accu-Chek active glucometer and recorded. On each clinic visit, when patients arrived at the clinic, before they went to see the physician a short consent form was handed to them right after they were informed and made aware of the ongoing study and how it would benefit them. Patients who consented and met the study inclusion criteria remained behind after seeing the physician for a series of small tests and blood was drawn for laboratory analysis. Each patient was given a unique ID number that identified and linked the patient and the blood sample drawn. 4 mL of venous blood was collected from patients and control

subjects. Blood sample are centrifuged the same day using Ultra-Vari 8 high powered centrifuge at 2,000 RCF (3,300 RPM) for 10 min. Blood plasma was collected in a sterile crial vial tube and labeled with patient code initials and study number. Plasma was stored at 2–8 °C for next day analysis. Oxidative stress was determined through an indirect measurement of lipid peroxide levels as thiobarbituric acid reactive species (TBARS). For the analysis of TBARS levels, Zeptometrix's TBARS assay kit supplied by Helvitica health care Sarl, Geneva, Switzerland was used. Malondialdehyde (MDA) level is used as an index of lipid peroxidation, which reacts with thiobarbituric acid to give a pink coloured product whose concentration was determined from optical density measurement at 532 nm using UV–VIS spectrophotometer.

Results

The majority of diabetic patients enrolled in our study i.e., 50/80 (62.5 %) were hyperglycemic with FBG level >10 mmol/L. The WHR for 56/80 (70 %) of the patients were in the high risk range and 54/80 (67.5 %) of them were either overweight or obese. The FBG level for control subjects was in the normal range with only one having FBG level of <5 mmol/L. However two-third of the controls (20/30) were also overweight. One-third of the patients admitted that they consume alcohol and 9 (11 %) are smokers. Fifty percent of the patients said they do physical exercise for different time duration. It was found out that 48(60 %) of the patient population were known hypertensive. Lipid profiles of the study population were also determined and the findings are being processed for publication. From patient's history duration of stay with the disease after being diagnosed as diabetic was recorded and 33/80 (41.2 %) have stayed >10 years with the disease (Table 1).

The mean ($\pm$ SD) value of plasma lipid peroxide for the control group was 31.36 ± 7.53 and the mean plasma lipid peroxide value for Type 1 diabetic patients was 70.56 ± 44.18 while for Type 2 diabetic patient the mean plasma lipid peroxide level was 53.27 ± 25.24 . Patients with peripheral vascular (PVD) disease complication had high level of lipid peroxide compared to patient without PVD (57.18 ± 3.02 versus 52.16 ± 2.63). However, the observed difference was not statistically significant as shown in Table 2 below.

The diabetic group mean ($\pm$ SD) value for P.TBARS (55.86 ± 29.17) was found to be high compared to the control group P.TBARS mean value (31.36 ± 7.53). The observed difference was statistically significant (Table 3).

A significant correlation between lipid peroxide and fasting blood glucose was observed ($p < 0.01$) for the diabetic group. In addition, a significant correlation existed between lipid peroxidation and BMI ($p < 0.05$) for the

Table 1 Demographic and baseline clinical data for diabetic patients

Parameters	Total (<i>n</i> = 80)	Percentage (%)
Duration of diabetes (years)		
1–10	47	58.8
11–20	24	30.0
21–30	9	11.2
Known hypertensive	48	60
BMI (Kg/m ²)		
<18.50	3	3.8
18.50–24.99	23	28.8
25–29.99	32	40.0
30+	22	27.5
W/H ratio		
Low risk	12	15
Moderate risk	12	15
High risk	56	70
FBG		
<6 mmol/L	4	5
6.1–9.9 mmol/L	26	32.5
>10 mmol/L	50	62.5
Age group		
25–45	21	26.2
46–66	47	58.8
>66	12	15
Sex		
Males (M)	23	28.8
Females (F)	57	71.3

Table 2 Lipid peroxide levels in various diabetes mellitus groups

Groups	Total	P.TBARS (nmol/mL) ± SD	<i>p</i> value
Type 1 DM	12	70.56 ± 44.18	NS
Type 2 DM	68	53.27 ± 25.24	NS
DM with complication	59	57.18 ± 3.02	NS
DM without complication	21	52.16 ± 2.63	NS

Independent sample *t* test: *p* > 0.05 not significant (NS)

diabetic group. Also in the case of controls, a significant correlation was observed between lipid peroxide and LDL cholesterol and FBG (*p* < 0.05) as shown in Table 4. There was a statistically significant positive correlation between plasma lipid peroxides level and the cardio vascular disease risk ratio indices (*r* = 0.0222, *p* < 0.05) (Table 5).

Discussion

Diabetes is no more a disease of the developed world as the number of diabetic cases is constantly increasing even in the developing world. One of the devastating conditions of

Table 3 Mean values of lipid fractions and lipid peroxide (TBARS)

Lipid parameters	Diabetics(<i>n</i> = 80)	Controls(<i>n</i> = 30)	<i>p</i> value
Total cholesterol (mmol/L)	5.16 ± 1.21	4.73 ± 1.07	NS
Triglycerides (mmol/L)	1.52 ± 0.65	1.66 ± 1.28	NS
HDL cholesterol (mmol/L)	1.16 ± 0.40	1.18 ± 0.56	NS
LDL cholesterol (mmol/L)	3.68 ± 1.19	3.27 ± 0.94	NS
P.TBARS (nmol/mL)	55.86 ± 29.17 ^a	31.36 ± 7.53	0.001

Independent sample *t* test: ^a*p* < 0.001 and NS = not significant, means shown as ± SD

Table 4 Correlations between FBG, BMI, lipid variables and lipid peroxidation

Variables	Diabetic patients, <i>r</i>	Control subject, <i>r</i>
Total cholesterol (TC)	0.11	0.10
Triglycerides	0.04	0.07
HDL cholesterol	−0.05	0.24
LDL cholesterol	0.12	0.37 ^b
FBG	0.34 ^a	0.42 ^b
BMI	−0.26 ^b	0.12

Values are Pearson's correlation coefficient (*r*) ^a*p* < 0.01 and ^b*p* < 0.05

Table 5 Correlation between P.TBARS, TC/HDL and LDL/HDL Ratios

Variable	Mean ± SD	Correlation coefficient (<i>r</i>)	<i>p</i> value
TC/HDL ratio	5.08 ± 3.4	0.222	0.05
LDL/HDL ratio	3.77 ± 3.2	0.223	0.05

diabetes is oxidative stress. Oxidative stress is a condition that occurs when there is an imbalance between production of free radicals and their scavenging in disease conditions. Increased oxidative stress has been observed in severe diabetic patients indicated by increased free radical production [10], lipid peroxidation, and diminished antioxidant status [11]. Oxygen free radicals generated through auto-oxidation of glucose in uncontrolled diabetes, are implicated in the pathogenesis of diabetes mellitus [12]. These oxygen free radicals (OFR), when beyond the ability of the cells antioxidant power, initiate membrane lipid peroxidation resulting in cell damage and tissue injury [13]. Under normal circumstances, the OFRs are cleared by the body's antioxidant system but during diabetes this inherent biological system becomes compromised. Hence the chances of cell damage and injury are increased, which

could lead to endothelial damage and atherosclerosis [14]. Our present study on Type 1 and Type 2 diabetic patients is also in agreement with previous reports. Due to hyperglycemia, patients seem to have increased lipid peroxidation that led to diabetic complications. 62.5 % of the patients were hyperglycemic (Table 1) and the mean plasma lipid peroxide levels were very much raised in diabetic patients as compared to controls and the difference was statistically significant (Table 2).

A positive correlation was observed between lipid peroxide levels and diabetic complication, which indicates that increased OFR leading to oxidative stress is a *sine qua non* of complications in diabetes. Type II diabetics had higher mean serum lipid peroxide levels than Type I patients and also patients with complication had higher serum lipid peroxide levels but the difference was not statistically different (Table 3). Accordingly Type 2 patients will be more prone to develop complications like cataract, foot ulcer, hypertension, nerve ending problems and atherosclerosis more than the latter. There was a statistical correlation between FBG levels, BMI and lipid fractions with lipid peroxide levels (Table 4). This indicates that the core problem during diabetes is poor glycaemic control, which leads to protein glycation, lipid peroxidation, oxidative stress and finally to varieties of complications. Lipid fractions, that have an effect in development of diabetic complications, also correlate with lipid peroxide levels (Table 5).

Increased LDL but lowered HDL levels and on the other hand increased cholesterol and triglyceride but decreased HDL level indicates deranged lipid profiles which also contribute to the complication in diabetes. All the diabetic patients enrolled in our study were not taking any statin drugs for control of lipid profiles but most of our patients had a deranged lipid profile, which we have not shown the data here. The patients also had uncontrolled blood glucose level. These two scenarios evidently contribute for the oxidative stress and the clinical complications like hypertension, which was common in many of the patients (60 %). This implies that despite the fact that patients have follow-up, they have poorly controlled blood glucose level and deranged lipid profile. This may be either patients do not adhere to the advice and regimens given to them by their physicians or there is no strict and timely follow up by physicians due to inexplicable reasons. Most of the patients (67.5 %) were either overweight or obese, and this tallies with the conditions of diabetes. This is an indication that the patients are not controlling their dietary habit hence

they are prone for complications. The number of Type 1 diabetics was comparatively small in our study and we capitalized on correlative analysis and this could bring a limitation in our interpretation and conclusion. However, our findings were consistent with previous works done in this area.

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