Original Article

Oxidative stress and glycaemic control determinants in Type 2 DM

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ABSTRACT

***Objective**:* Diabetes mellitus has been associated with increased risk of oxidative stress. There is limited information on the significance of an early marker of oxidative stress which can reflect the total antioxidative activity, especially in poorly controlled diabetes mellitus. The aim of this study was to establish association of glycaemic control determinants and total antioxidant activity and also to evaluate the frequency of occurrence of reduced antioxidant activity in poorly controlled glycaemia.

***Methodology**:* This was a cross sectional study carried over three months. The study population consisted of two hundred type 2 diabetes mellitus patients attending the diabetic clinics of Lagos State University Teaching Hospital, Ikeja and General Hospital, Gbagada. These categories of patients were males and females between the ages of 40 and 60 years. Glycaemic control was assessed using fasting plasma glucose, fructosamine and glycosylated haemoglobin. Biochemical parameters were compared using students't test, Pearson's correlation coefficient and analysis of variance.

Results: This study demonstrated reduced total antioxidant activity in Nigerian diabetics in comparison with control subjects ($p < 0.05$) and was observed to be much lower in complicated diabetes mellitus patients. Consistent negative association of total antioxidant activity with short, medium and long term glycaemic control determinants fasting plasma glucose "r = -0.43, p = 0.001", fructosamine ''r = -0.42, p = 0.002'' and glycosylated haemoglobin "r = - 0.35, p = 0.030" was observed.

Conclusion: The clinical usefulness of total antioxidant activity as a surrogate marker of glycaemic control is shown. This may be useful in the early detection of diabetic complications. Significant reduction of total antioxidant activity especially among diabetics with complications suggests a possible role of this in the pathogenesis of diabetic complications.

KEY WORDS: Oxidative stress, Glycaemic control, T2 Diabetes Mellitus.

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INTRODUCTION

Oxidative stress is thought to contribute to the development of a wide range of diseases, including the pathologies caused by diabetes mellitus.[1] In many of these diseases, it is unclear if oxidants trigger the disease or if they are produced as a consequence of the disease and cause the disease symptoms. The relative importance and interactions between these antioxidants is very complex, with the various

metabolites and enzyme systems having synergistic and interdependent effects on one another.[2,3]

The action of one antioxidant may depend on the proper function of other members of the antioxidant system.[4] Oxidative stress arises when the imbalance between reactive oxygen species (free radicals) and the antioxidant system is in favour of the former.[5] Hypothetically, it would be expected that the free radicals generated as a result of hyperglycaemia in diabetes mellitus will overwhelm the activity of antioxidants. These free radicals react with organic materials and phospholipids to cause lipid peroxidation, fragmentation of proteins and deamination of guanine and adenine in DNA chain to cause gene mutation and cell damage.[6]

Studies have shown increased malondialdehyde (MDA) – a product of lipid peroxidation, consequent to oxidative damage in non controlled type 2 diabetes mellitus (T2 DM) as well as reduced levels of alpha tocopherol, a form of nutritional antioxidant in non controlled T2 DM subjects compared with controlled ones.[7] Van der jast et al, 2001 also reported increased lipid peroxidation, which was detected in the early stages of T2 DM.[8] Nevertheless, information on the association of the total antioxidant activity with indices of short, medium and long term glycaemic control is not substansive. This gives a reflection of the dynamic equilibrium that is influenced by interactions between each serum antioxidative constituents and not just the sum of the various antioxidative substances.

The aim of this study was to evaluate an association between total antioxidant activity with short (fasting blood glucose), medium (fructosamine) and long term (glycosylated haemoglobin) glycaemic control indices and also to evaluate the frequency of occurrence of reduced antioxidant activity in poorly controlled glycaemia. Other objective was to establish a reference range of total antioxidant activity among healthy Nigerians.

METHODOLOGY

This was a cross sectional study carried over three months. The study population consisted of two hundred type 2 diabetes mellitus patients attending the diabetic clinics of Lagos State University Teaching Hospital, Ikeja and General Hospital, Gbagada. These are the two largest DM centres in Lagos State, a cosmopolitan city in the south western region of Nigeria. These categories of patients were males and females between the ages of 40 and 60 years, the age range where most T2 DM commence. We considered it necessary not to include T2DM over 60 years, as ageing has been associated with increased free radical load.[9]

Ethical approval was received from the Ethics and Research committee before commencement of the study. Informed consent of the patients was gotten through the aid of a well structured questionnaire. The inclusion criteria for DM patients were those with post / present history of oral hypoglycaemic agents, singly or in combination with insulin or history suggestive of beta cell failure for those on sole insulin treatment. The control samples were one hundred apparently healthy age matched males and females, recruited from the staff of the Hospital. In a bid to ascertain that the control groups were not DM patients, who had well controlled DM, they were questioned on the use of medications. Fasting Blood Samples were collected into fluoride oxalate, lithium heparin and EDTA bottles.

Laboratory analysis: Fasting plasma glucose was done spectrophotometrically using the glucose oxidase method while glycosylated haemoglobin (HbA1c) was estimated by spectrophotometric - chromatographic ion exchange method using Biosystems kit, Spain. For Diabetics, a value of HbA1c between 6.7 – 7.3% is the targeted goal, while HbA1c of 7.3 – 9.1 % is regarded as good control. Values above 9.1% require immediate action.[10] Fructosamine was estimated turbidimetrically using Fortress kit, UK. A reference range of 205 - 285µmol/l for adults without diabetics is recommended, values above 396umol/l indicate poor control.[11] Total antioxidant activity was estimated by the method of Koracevic et al, 2001.[12]

Principle – A standardized solution of Fe-EDTA complex reacts with hydrogen peroxides by a fenton type reaction, leading to the formation of hydroxyl radicals (OH). These reactive oxygen species degrade benzoate, resulting in the release of thiobarbituric acid reactive substances (TBARS).[13,14] Antioxidants from the added sample of human fluid cause suppression of the production of TBARS. This reaction can be measured spectrophotometrically and inhibition of colour development defined as antioxidant activity.

RESULTS

The DM free status of the healthy controls was ascertained by having them subjected to glycosylated haemoglobin and fasting plasma glucose tests. They were deemed as not having DM if the Fasting plasma glucose and HbA1c were less than 100mg% and 5.7% respectively.[15] A total number of 81 (40.5%) of the

Table-I: Means± SEM of fasting blood glucose, fructosamine and glycosylated haemoglobin of diabetic patients with controls.

Variables	*Patientsn = 200*	*Controlsn = 100*	*t value*	*p value*
Fasting blood glucose (mg/dl)	162.73± 5.00	92.59± 1.44	9.852	0.000 *
Fructosamine (µmol/l)	334.91± 11.42	225.0± 5.02	6.662	0.000 *
Glycosylated haemoglobin (%)	6.35± 0.17	5.11± 0.09	5.035	0.001 *

n = number of subjects SEM = Standard error of mean *= p < 0.05 (Significant)

T2DM were without any complications, while presence of complications was observed in 119 (59.5%). The prevalence of various microvascular complications observed were retinopathy (20%), neuropathy (31%), nephropathy with renal failure (1%) and foot ulcers (7.5%).

A reference range using mean ± 2 standard deviation in the healthy Nigerians was ''1.16 – 1.64 mmol/l''. The mean ± SEM of total antioxidant activity differed significantly between type 2 diabetic patients and the control subjects ''0.97±0.02mmol/l vs 1.40±0.02 mmol/l,p=0.000''. Similarly, a much lower value of total antioxidant activity was observed in complicated diabetic patients ''0.95±0.031mmol/l,p=0.000''. Within group and between group comparison using analysis of variance was highly significant ''f =98.815, p=0.000''.

Pearson's correlation coefficient showed an inverse and significant association between indices of glycaemic control and total antioxidant activity. A moderate association ''r = -0.43, p = 0.001'' was observed with fasting plasma glucose and total antioxidant activity. Moderate inverse association ''r =-0.42, p=0.001'' was observed with fructosamine, as well as HbA1c ''r = -0.35, p = 0.030''. Poor glycaemic control is associated with reduced antioxidant activity.

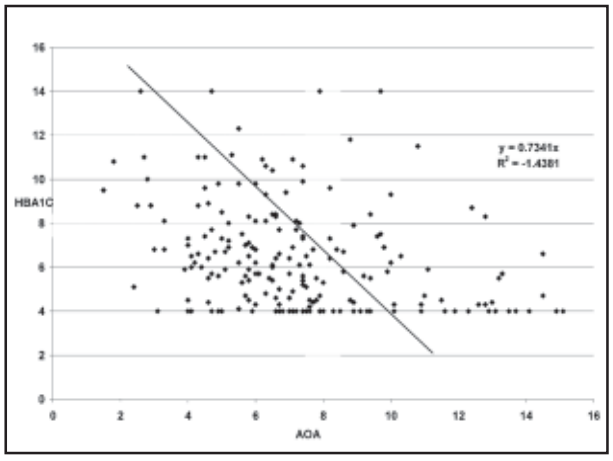

Fig-1: Scattergram shows relationship between total antioxidant activity (AOA) with glycosylated haemoglobin (HbA1c). A graphical representation between AOA and glycosylated haemoglobin is shown in Fig-1. An inverse relationship between AOA and glycosylated haemoglobin was observed ''r = -0.40, p = 0.001''. Poor glycaemic control is associated with reduced AOA.

DISCUSSION

This study demonstrated reduced total antioxidant activity may play a role in the pathogenesis of diabetic complications. A depletion of antioxidant activity was associated with poor glycaemic control, both during short, medium and long term assessment. Our findings agree with other studies which observed a similar pattern only with long term glycaemic control.[16] Maintaining good glycaemic significantly lowers the incidence of complications from DM.[17] The prevalence of various microvascular complications observed in this study is in contrast with what was earlier reported up in the early 1990s in sub Saharan Africa, which gave widely variable figures. The figures range from 9 – 16% for cataracts, 7

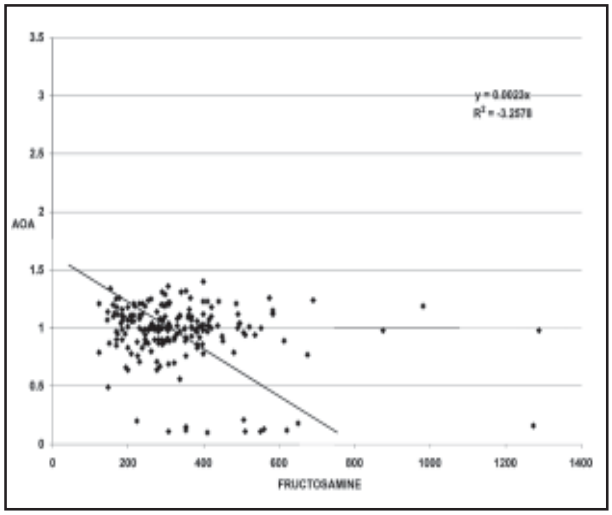

Fig-2: Scattergram showing relationship between total antioxidant activity with fructosamine. An association between total antioxidant activity with fructosamine levels is shown in Fig-2 An inverse relationship (r=-0.42, p= 0.002) was observed. Poor glycaemic control is associated with reduced free radical scavengers (antioxidant activity)

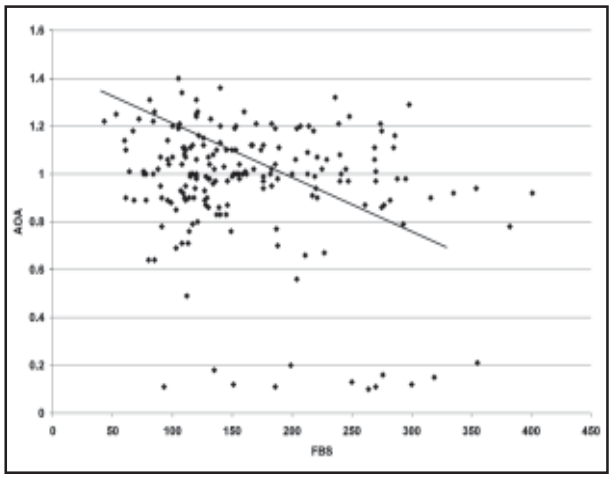

Fig-3: Scattergram showing relationship between total antioxidant activity with fasting blood glucose. The graph (Fig-3) shows the relationship between total antioxidant activity and fasting plasms glucose. An inverse relationship was observed between antioxidant activity and fasting plasma glucose (r = - 0.43, p = 0.001). Excess free radicals generated as a result of hyperglycaemia overwhelms the activity of the antioxidants.

– 52% for retinopathy, 1 – 5% for retinopathy, 6 – 47% for neuropathy, 6 – 30% for nephropathy, 1 – 5% for macroangiopathy.[18,19]

We have also shown much lower levels of total antioxidant activity in T2DM in complicated T2DM. Our findings in relation to complications somehow agrees with Sawant et al, 2007, although there study only involved markers of oxidative stress and not the total activity of the antioxidants. In their report, increased lipid peroxidation and reduced antioxidant vitamin c status in complicated T2DM was observed with a mean percentage rise in MDA levels in complicated T2DM of 130%, while vitamin c showed a mean percentage reduction of 45% in complicated T2 DM. markers of oxidative damage was also reported to show a significant positive correlation of MDA levels and HbA1c and inverse correlation of vitamin c and HbA1c.[20]

In this study, we observed a lower reference range for total antioxidant activity among healthy Nigerians as compared with what was earlier reported for Caucasians.[21] The poor nutritional status in sub Saharan Africa may contribute to the low antioxidant activity, especially with regards to the impact of nutritional antioxidants. The frequency of occurrence of reduced total antioxidant activity in T2DM with poor - short glyceamic control (fasting plasma glucose greater than 125mg/dl) was 80%, medium glycaemic control (fructosamine - greater than 396umol/l was 85%), while long term glycaemic control (HbA1c > 9.1% was 85.9%). Total antioxidant activity could thus serve a useful surrogate marker of glycaemic control assessment.

These findings point to the fact that T2DM with poorly controlled glycaemia are more prone to oxidative stress. This indicates that poor glycaemic control is associated with reduced free radical scavengers in T2DM. By implication, improved glycaemic control may preserve serum antioxidant status in T2DM. Increased formation of reactive oxygen species and / or decreased antioxidant system is widely recognized as an important feature in many diseases. Cells and biological fluids have an array of protective antioxidant mechanisms, both for preventing the production of free radicals and for repairing oxidative damage.[22] Predisposition of T2DM to complications due to reduced antioxidant activity could be attributed to suppression of interferon gamma by excess free radicals.[23]

Interferon gamma, otherwise known as type 2 interferon or immune interferon is an immune modulator and offers greater protection to healthy subjects.[24] Plasma fructosamine levels, otherwise known as glycated albumin, aid in appraising glycaemic control for about three weeks. This is based on the half life of albumin. Glycosylated haemoglobin on the other hand, assesses glycaemic control for four months as a result of the life span of red blood cells. As a result, changes in fructosamine values alert the physician to deteriorating glycaemic control earlier than in HbA1c, which also reflect depleted activity of antioxidants.

CONCLUSION

Consistent negative association of total antioxidant activity with glycaemic control determinants of over three weeks and over four months indicates the clinical usefulness of total antioxidant activity as a surrogate marker in the early detection of diabetic complications due to oxidative stress.

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Author's Contribution:

AA designed the study, participated in the data collation, statistical analysis, funding and writing the draft of the manuscript.
AOO participated in the study, subjects' selection, funding and data collation.
OEO participated in the study, funding and data collection of data.
OJC participated in the study and data collection.

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