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Decreased total antioxidant levels and increased oxidative stress in South African type 2 diabetes mellitus patients

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Background: Chronic hyperglycaemia in diabetes mellitus leads to increased lipid peroxidation in the body, followed by the development of chronic complications due to oxidative stress. **Objective:** The aim of this study was to compare total antioxidant (TAO) levels and oxidative stress in type 2 diabetes mellitus (T2DM) patients with that of healthy controls without diabetes. **Methods:** A total of 98 participants (57 T2DM and 41 healthy people) gave their consent and participated in the study. Routine biochemical methods were used for fasting plasma glucose (FPG), glycosylated haemoglobin (HbA1c) and lipid profile measurements. Serum TAO levels, malondialdehyde (MDA), oxidised low-density lipoprotein (ox-LDL) levels and superoxide dismutase (SOD) activity were analysed using standard commercial reagent kits. **Results:** A significant rise in FPG, HbA1c, triglycerides, MDA and ox-LDL, and a significant reduction in TAO and high-density lipoprotein cholesterol (HDL-C) was observed in T2DM patients compared with controls. A significant negative relationship was observed between TAO levels and MDA levels in the T2DM group. Increased lipid peroxidation and reduced antioxidant levels were observed in T2DM patients. **Conclusion:** Early management through an antioxidant-rich diet and lifestyle changes in T2DM patients would help to avert the debilitating complications of diabetes.

Keywords: diabetes mellitus, oxidative stress, total antioxidant status

Introduction

Diabetes mellitus (DM) is a cluster of metabolic disorders characterised by abnormally elevated blood glucose levels (hyperglycaemia),¹ which arise from the body's inability to produce insulin or to use it to its full potential.² DM is a lifetime progressive metabolic disease and, according to recent estimates for the year 2015, 415 million adults aged 20–79 suffer from the disease worldwide.³ These estimates include 193 million people who are undiagnosed and by the year 2040, if the current rate of increase in cases continues, 642 million people will be suffering from diabetes.³ DM is the fourth primary reason for death by disease universally and has become a challenging health problem for the twenty-first century.^{2,4}

The incidence of DM is rising at an alarming rate; mainly the type 2 (non-insulin dependent diabetes mellitus (NIDDM)).^{5,6} Sub-Saharan Africa, including South Africa, now faces a double burden of disease, with an epidemiological transition from transmissible diseases to non-communicable or 'lifestyle' diseases.^{7,8} The prevalence study by Bertram *et al.* reported a potential rise in type 2 diabetes mellitus in South Africa from 5.5% in 2000 to 9% in 2009 in people aged 30 or older since the previous approximations.⁹ Secondary studies by Bertram *et al.* furthermore revealed that in South Africa around 55% of cases remained undiagnosed.⁹ In a cross-sectional survey of 642 participants aged ≥ 31 years from an urban South African coloured community in Bellville South, Cape Town, T2DM was evaluated using the World Health Organization (WHO) criteria. The crude prevalence of T2DM was 28.2%, and undiagnosed T2DM was present in 18.1% of participants.¹⁰ In a recent report by International Diabetic Federation (IDF) on the prevalence of diabetes in African countries, South Africa was recorded as having an average 2.3 (1.2–4.6) million people with diabetes.³

Several pathological processes are involved in the development of diabetes, which range from autoimmune obliteration of the beta cell of the pancreas causing insulin deficiency (type 1 diabetes mellitus) to abnormalities that result in insulin resistance (T2DM).¹ As a result of chronic hyperglycaemia and insulin resistance, various long-term complications of diabetes develop. These complications include both microvascular and macrovascular irregularities, such as retinopathy, peripheral neuropathy, autonomic neuropathy, cardiovascular symptoms and nephropathy.^{1,11} Oxidative stress is accountable for the development of chronic complications of DM¹² and results from chronic hyperglycaemia, dyslipidaemia and elevated fatty acids in the circulation.¹³

Oxidative stress is an inevitable consequence of life in an atmosphere that is oxygen-rich¹⁴ and arises when a synthesis of reactive oxygen species (ROS) and reactive nitrogen species (RNS) surpasses the capacity of cellular antioxidant defences to eradicate these toxic species. Examples of ROS are superoxide, hydroxyl radical, peroxy radical, and hydroperoxyl radical. The non-radical ROS are hydrogen peroxide and hypochlorous acid. Examples of RNS include nitric oxide, nitrogen dioxide, peroxynitrite and, nitrous oxide.^{15,16} Most of the free radicals are produced in small amounts under normal physiological conditions and scavenged by the endogenous antioxidant system. The antioxidant system includes enzymes like SOD, catalase, glutathione peroxidase and molecules like GSH, uric acid, bilirubin, lipoic acid, albumin, transferrin, vitamin E, vitamin C, carotenoid, copper and zinc.¹⁷ The antioxidants scavenge the free radicals by several mechanisms. The enzymes degrade free radicals; proteins such as transferrin can bind to metals that stimulate the synthesis of free radicals; and vitamins C and E act as free radical scavengers.¹¹ Vitamin C is a water-soluble molecule and usually scavenges hydroxyl radicals, while vitamin E, a lipid soluble vitamin, interferes with chain reactions of lipid peroxidations.¹¹

In diabetes and other pathological conditions this defence mechanism is altered, and ineffective scavenging of ROS and RNS plays a vital role in causing tissue damage in the diabetic patient.¹⁸ The increased prevalence of free radicals results in the activation of stress-signalling pathways and drains both enzymatic and non-enzymatic antioxidants, having a negative impact on the quality of life and lifespan of the patient. ROS and RNS play a role in multiple disease conditions including diabetes and its complications.¹⁹

Several other mechanisms are also implicated in the generation of oxidative stress due to hyperglycaemia in diabetes. These mechanisms include glucose auto-oxidation, increased glucose flux through the polyol pathway, non-enzymatic and progressive glycation of proteins and the formation of advanced glycosylation end products (AGEs).²⁰ An increase in the polyol pathway depletes the NADPH, which is utilised by the enzyme aldose reductase that catalyses the conversion of glucose to sorbitol.²

Numerous studies have reported increased oxidative stress in type 2 diabetes mellitus patients compared with their healthy counterparts.^{21,22} In Africa, studies on oxidative stress and diabetes have also reported similar findings.^{23,24} There are very few to no studies conducted on oxidative stress levels in South African people with type 2 diabetes. This investigation aimed to investigate the oxidative stress and total antioxidant levels in South African T2DM patients compared with healthy volunteers without diabetes.

Materials and methods

Ethical clearance was acquired from the Research Ethics and Biosafety Committee, the Faculty of Health Sciences, Walter Sisulu University (Bioethics clearance No: 012/012).

Research participant recruitment

Inclusion criteria: The cross-sectional observational study was conducted on total of 98 participants (57 black, South African, known T2DM patients attending selected diabetes clinics and 41 healthy volunteers as controls) within the age group 35–75 years, all of Xhosa ethnicity, in Mthatha, Eastern Cape Province of South Africa.

Exclusion criteria: Patients who have HIV infection and other chronic diseases such as tuberculosis, thyroid problems, coronary artery diseases, renal complications and other complications of diabetes, if reported, were not included in this study.

The clinical examination of the T2DM patients was carried out by the clinician of the diabetes clinic. Control participants were selected randomly from the general population, keeping in mind the selection criteria. The participants were sensitised concerning the study, and those who volunteered were asked to sign an informed consent form before blood collection. A questionnaire was used to obtain anthropometric data as well general information (medication, physical activity, family history, duration of diabetes and other chronic illnesses) from each participant.

Sample collection and preparation

Fasting blood (approx. 20 ml) was collected in vacutainer tubes from each participant. Plasma was separated from the specimen within three hours of collection. Serum samples were stored at –70 °C if not analysed on the day of collection and used within a month. Fasting blood samples were used for measurements of plasma glucose, glycated haemoglobin (HbA1c), lipid profile, TAO level, malondialdehyde (MDA), thiobarbituric acid reactive

substances (TBARS), oxidised LDL (ox-LDL) levels and superoxide dismutase (SOD) enzyme activity.

Methods

Plasma glucose, HbA1c and lipid profile were measured by routine methods, using the Roche cobas® 6000 chemical auto-analyser (Roche Diagnostics USA, Indianapolis, IN, USA), by NHLS (National Health Laboratory Services) of NMAH (Nelson Mandela Academic Hospital). Different methods for assessing lipid peroxidation and total antioxidant activity for measuring the oxidative stress in diseases have been discussed by various authors.²⁵ In this study the TAO level in serum was measured using a Sigma-Aldrich kit (ABTS method; Saint Louis, MO, USA). MDA (TBARS) and SOD enzyme activity were measured using Cayman assay kits (colorimetric method; Cayman Chemical Company, Ann Arbor, MI, USA) and ox-LDL was measured by the ELISA technique using a Mercodia kit (Uppsala, Sweden). The BioTek KC₄ Autoreader (BioTek Instruments, Inc., Winooski, VT, USA) was used for all the analyses referred to above.

Statistical analysis

Statistical analysis was conducted using the IBM Statistical Package for the Social Sciences, version 23 (IBM Corp, Armonk, NY, USA). Data are expressed as mean ± SD and median (IQR) depending on their distribution (normal and not normal) respectively. The statistical significance of differences between the means of quantitative variables across groups was evaluated by Student's t-test for normally distributed data, and the non-parametric Mann-Whitney U test was used for parameters violating normal distribution. The bivariate correlations were ascertained using Spearman rank correlation to analyse relationships between continuous variables.

Results

Table 1 presents information regarding the general characteristics of participants. The mean age was comparable across groups and showed no significant difference ($p = 0.101$). The mean BMI for both groups was high, with T2DM patients having higher BMIs compared with individuals in the healthy control group; the difference was not, however, statistically significant ($p = 0.378$). Biochemical characteristics of participants are presented in Table 2. FPG and HbA1c were significantly higher in T2DM patients compared with those of persons in the control group ($p < 0.0005$ and $p < 0.0005$ respectively). Serum triglyceride (TG) levels were substantially higher in the T2DM group as opposed to the controls ($p = 0.024$), and high density lipoprotein cholesterol (HDL-C) was significantly different between groups ($p = 0.008$), with the T2DM patients having lower HDL-C compared with the persons in the healthy control group. There was no difference in total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) between the two groups.

Serum TAO, MDA (TBARS) and ox-LDL levels were significantly different in both groups. A statistically significant reduction in

Table 1: Demographic characteristics of diabetic and control participants

Parameters	Control group, <i>n</i> = 41	T2DM group, <i>n</i> = 57	<i>p</i> -value
Age (years)	54.24 ± 6.05	56.61 ± 7.59	0.101
Weight (kg)	76.0 (87.8–65.7)	80.0 (96.0–70.0)	0.125
Height (cm)	159.4 ± 6.20	162.1 ± 7.87	0.067
BMI (kg/m ²)	30.4 ± 7.28	31.6 ± 6.49	0.378
Duration of diabetes (years)	–	6.87 ± 6.34	

Notes: Data are shown as mean ± SD and median (IQR), BMI = body mass index.

Table 2: Biochemical characteristics of diabetic and control participants

Parameters	Control group, n = 41	T2DM group, n = 57	p-value
FPG (mmol/L)	4.9 (5.2 – 4.6)	8.1(10.7–5.90)	< 0.0005**
HbA1c (%)	6.0 (6.2–5.8)	8.5 (10.2–6.9)	< 0.0005**
Haemoglobin (g/dl)	14.1 ± 1.49	14.1 ± 1.44	0.854
TC (mmol/L)	4.70 ± 0.92	4.51 ± 1.23	0.420
TG (mmol/L)	1.10 (1.55–0.90)	1.30 (2.05–1.07)	0.024*
HDL-C (mmol/L)	1.40 (1.55–1.10)	1.16 (1.40–1.0)	0.008*
LDL-C (mmol/L)	2.72 ± 0.88	2.57 ± 1.03	0.461

Notes: Data are shown as mean ± SD and median (IQR), FPG = fasting plasma glucose; HbA1c = glycated haemoglobin, TC = total cholesterol; TG = triglyceride; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol. The median and mean difference is significant at * $p < 0.05$ and ** $p < 0.005$ level.

TAO levels was observed in the T2DM patients compared with healthy participants ($p = 0.017$), whereas median MDA and ox-LDL levels were significantly raised in patients with diabetes compared with controls. There was no significant difference in total SOD activity between the two groups, as shown in Table 3.

An analysis of the correlations between HbA1c, MDA, ox-LDL, SOD and TAO in both groups is shown in Table 4. There was a significant negative correlation between TAO levels and MDA in the T2DM group ($r = 0.291$, $p = 0.029$). There was also a significant negative correlation between HDL-C and HbA1c, TG and ox-LDL levels in the control group, whereas in the T2DM patients only TG levels were significantly associated with HDL-C, as presented in Table 5.

Table 3: Antioxidant status and lipid peroxidation in diabetic and control participants

Parameters	Control group, n = 41	T2DM group, n = 57	p-value
TAO (mM)	0.60 (0.80–0.44)	0.48 (0.61–0.37)	0.017*
Total SOD activity (U/ml)	3.19 (4.68–2.53)	4.01 (5.26–3.0)	0.096
MDA (μM)	3.43 (4.75–2.42)	4.33 (5.75–3.29)	0.004**
Ox-LDL (U/L)	77.5 (107.4–56.2)	95.9 (137.6–65.2)	0.022*

Notes: Data are shown as mean ± SD and median (IQR). The mean and median difference is significant at * $p < 0.05$, and ** $p < 0.005$ level. TAO = total antioxidant; SOD = superoxide dismutase; MDA = malondialdehyde; ox-LDL = oxidised LDL.

Table 4: Spearman's correlation between HbA1c, MDA, ox-LDL and SOD activity and TAO level in diabetic and control participants

Groups	MDA r-value	Total SOD r-value	Ox-LDL r-value	HbA1c r-value
TAO in control group	0.141	–0.286	–0.053	–0.221
TAO in T2DM group	–0.291*	–0.251	–0.229	–0.103

Notes: Data are presented as Spearman correlation coefficient (r), * $p < 0.05$. MDA = malondialdehyde; SOD = superoxide dismutase; ox-LDL = oxidised LDL; HbA1c = glycated haemoglobin; TAO = total antioxidant.

Table 5: Spearman's correlation between HbA1c, triglyceride, ox-LDL levels and HDL cholesterol in diabetic and control participants

Groups	Triglyceride r-value	Ox-LDL r-value	HbA1c r-value
HDL-C in control group	–0.361*	–0.594**	–0.325*
HDL-C in T2DM group	–0.391*	–0.087	0.047

Notes: Data are presented as Spearman correlation coefficient (r), * $p < 0.05$ and ** $p < 0.005$ levels. Ox-LDL = oxidised LDL; HbA1c = glycated haemoglobin; HDL-C = high density lipoprotein cholesterol.

Discussion

In the present study there was a marked rise in HbA1c and FPG levels in T2DM patients compared with the control population, indicating excessive glycosylation of haemoglobin and poor control of diabetes as reported by other studies.^{23,26–29} An increase in the serum TG levels and a decrease in HDL-C levels were observed in patients with diabetes, indicating the presence of dyslipidaemia. Since the uptake of free fatty acids (FFAs) by the skeletal muscle and adipose tissue is mediated by insulin, an increase in insulin resistance would result in increased FFAs delivered to the liver. This leads to the overproduction of very low-density lipoprotein (VLDL) and increased VLDL-cholesterol concentrations, clinically manifesting as hypertriglyceridemia. An accumulation of the TG-rich lipoproteins in plasma can also result due to decreased lipoprotein lipase (LpL) activity.³⁰ Hypertriglyceridemia and reduced plasma HDL-C is commonly present in T2DM and has been reported by various studies.^{28,29,31}

Chronic hyperglycaemia, dyslipidaemia and elevated FFAs results in oxidative stress by stimulating ROS and RNS production,¹³ which attacks the lipids found in the plasma membranes, mitochondrial membranes and endoplasmic reticulum membranes and causes peroxidation.³² Once lipid peroxidation is initiated, propagation chain reactions will occur until termination products are produced, such as lipid hydroperoxide, which further decompose to aldehydes such as MDA and, 4-hydroxy-2 nonenal (4-HNE). Cyclic endoperoxides, isoprostanes and hydrocarbons may also be formed.^{18,33–38} The observed increase in MDA levels in T2DM patients in the present study is thought to be due to the increased production of lipid peroxides and their release in circulation, which would be consistent with previous studies.^{21–23,39} In the present study, increased oxidation of LDL particles was observed in the T2DM patients compared with the control group. Several factors, such as glycation, increased TG content and the reduced anti-oxidative properties of HDL are possible stimulators of LDL oxidation in T2DM patients.⁴⁰ TGs have an effect on LDL size and HDL-C inhibits the oxidative modification of LDL, so increased TG and decreased HDL-C can result in further oxidation of LDL in diabetes.²⁷ Similar observations have been reported by other studies.^{41–43}

A significant decrease in TAO levels among T2DM patients was observed in this study and has also been reported by various other authors in their studies.^{21,23,42} This reduction in TAO levels could be attributed to increased oxidative stress. This is evidenced by increased lipid peroxidation as well as by the excess utilisation of antioxidants against oxidative stress to minimise the damage. The significant negative correlation observed between TAO levels and MDA levels in the T2DM group in this study also explains the decrease in TAO levels with increased lipid peroxidation. Jamuna

Rani and Mythili showed a significant negative association between TAO levels and MDA levels in their study population.²¹ There was no significant difference in total SOD activity between the two groups, which concurs with the findings of Kesavulu *et al.* and Gurler *et al.* in their studies of T2DM patients.^{44,45}

A significant negative correlation was observed between HDL-C and serum TG in the T2DM patients and the control participants. In the T2DM group, no significant correlation was demonstrated between HDL-C and ox-LDL, but the control group showed a significant negative correlation. HDL-C contains enzymes such as paraoxonase, platelet activating factor, acetylhydrolase, and lecithin cholesterol acyltransferase and is involved in the reverse transport of cholesterol from the periphery to the liver. These enzymes prevent the formation of or metabolise the oxidised phospholipids. Evidence exists that HDL can reverse LDL oxidation by removing the oxidised phospholipids that make LDL harmful and lead to atherogenesis.⁴⁶ In the healthy control group, a significant negative association was observed between the HDL and LDL, which indicates that high HDL-C can decrease the oxidation of LDL. In the T2DM group, this correlation was not significant and could be due to the fact that in diabetes the anti-oxidative property of HDL is reduced. This reduction could be promoted by hyperglycaemia and the TG enrichment of lipoprotein.⁴⁰ This may be the reason why no correlation was observed between HDL-C and ox-LDL in the T2DM group.

Conclusion

The results of this study suggest that hyperglycaemia and dyslipidaemia seen in the T2DM patients could lead to an increase in lipid peroxidation and oxidative stress and thus a decrease in TAO levels. This decrease in TAO levels could be due to its increased utilisation in order to scavenge the free radicals produced in high amounts due to increased oxidative stress. Early intervention and a diet rich in antioxidants can reduce the risk of developing complications and increase the longevity and quality of life of patients with diabetes.

Limitations

The effects of diet and diabetic medication were not considered in the study.

Author contributions – All the authors contributed equally in the preparation of this manuscript.

Conflict of interests – The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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