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RESEARCH ARTICLE

Treatment of hyperglycaemia in newly diagnosed diabetic patients is associated with a reduction in oxidative stress and improvement in β-cell function

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Abstract

Background There exist several reports demonstrating enhancement in oxidative stress in diabetic patients; however, serial and comprehensive measurement of oxidative stress parameters in newly diagnosed diabetic patients is not yet reported. We measured the oxidative stress parameters in diabetic patients serially from the time of diagnosis and after starting treatment to study their association with glycaemia, insulin resistance and β-cell function.

Methods Fifty-four newly diagnosed diabetic patients were studied at diagnosis and 4 and 8 weeks after initiating anti-hyperglycaemic treatment. Oxidative stress parameters included activity of antioxidant enzymes, concentration of antioxidant molecules and damage markers. Oxidative stress score was computed as a collective measure of oxidative stress to interpret total oxidative stress state. Association of changing glucose levels with changing oxidative stress parameters over 8 weeks and association of oxidative stress score with insulin resistance and β-cell function was analysed by homeostasis model assessment (HOMA-IR and HOMA-β, respectively).

Results Eight weeks of treatment improved HbA_{1C} from 9.8 ± 2.1 to $7.7 \pm 1.0\%$. There was a significant increase in oxidative stress in diabetic patients [23.8 (95% CI 20.0, 27.6)] compared with non-diabetic subjects [-1.2 (-3.4, 0.9)] ($p < 0.001$). Non-diabetic subjects showed a stable status over 8 weeks. Improvement in hyperglycaemia in diabetic patients was associated with an improvement in oxidative stress parameters irrespective of the anti-diabetic treatment received. Oxidative stress score fell after 8 weeks and was significantly associated with an improvement in HOMA-β (standardized $\beta = -0.38$, $p < 0.01$) but not with HOMA-IR.

Conclusions Controlling hyperglycaemia in diabetic patients alleviates oxidative stress within 8 weeks of treatment, and improvement in oxidative stress parameters was related to an improved β-cell function.

Keywords antioxidant defence; beta-cell function; diabetes mellitus; glycaemic control; oxidative stress; oxidative stress score

Introduction

Oxidative stress refers to a state of imbalance between excess generation of reactive oxygen species (ROS) and inadequate antioxidant defence machinery

to combat it. Oxidative stress is known to be associated with many diseases such as diabetes mellitus, cancer, atherosclerosis and Alzheimer's disease [1].

Hyperglycaemia leads to excessive generation of free radicals, which overwhelm the antioxidant defence machinery of a cell, thereby generating oxidative stress. Intracellular ROS produced by proton electrochemical gradient results in increased production of superoxide [2]. Excessive production of free radicals in persistent hyperglycaemia is ascribed to autoxidation of glucose, non-enzymatic glycation of proteins and activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases and nitric oxide synthase; all of which produce ROS leading to oxidative stress [3,4]. This stimulates several biochemical pathways including polyol, hexosamine, advanced glycosylation end products and protein kinase C pathways [5], which contribute to the pathogenesis of complications in diabetes [6–10]. In addition to the role of oxidative stress in causing tissue damage, oxidative stress may be involved in the pathogenesis of diabetes by affecting insulin sensitivity and β-cell function. Recent studies in animals and pre-diabetic human beings have suggested a role for oxidative stress in the pathogenesis of insulin resistance [11,12]. Beta cells, known to intrinsically possess low activity of antioxidant enzymes, are vulnerable to oxidative damage [13]. Recent evidences suggest that β-cell loss in type 2 diabetes (T2D) results from a synergistic effect of glucotoxicity, endoplasmic stress and oxidative stress [14–16].

There are only a few studies on serial measurements of oxidative stress markers in T2D from the time of diagnosis and the effect of anti-hyperglycaemic treatment. We serially measured the antioxidant defence status as well as oxidative damage markers in diabetic patients from the time of diagnosis and compared these with serial measurements in healthy non-diabetic subjects. Further, we computed oxidative stress score taking into consideration different oxidative stress parameters and examined its correlation with glucose, insulin, HOMA-IR and HOMA-β.

Materials and methods

Subjects

Newly diagnosed T2D patients ($n = 54$) attending the Diabetes Unit, KEM Hospital, Pune (India), with fasting plasma glucose (FPG) concentration of >126 mg/dL (6.9 mmol/L) and healthy non-diabetic subjects ($n = 50$) with FPG concentration of ≤126 mg/dL (6.9 mmol/L) were studied. Non-diabetic subjects were healthy volunteers from academic institutions in Pune. Fasting blood samples were collected at baseline and at 4 and 8 weeks from both non-diabetic subjects and diabetic patients. Diabetic patients were put on anti-diabetic drugs to control hyperglycaemia as required and were advised diet and physical activity. Patients were also advised not to take any oral antioxidant supplements. The following types of subjects were excluded: pregnant women, individuals with excessive alcohol intake, chronic smokers, those receiving antioxidants, those with clinical infection and with inflammatory or malignant disease and those with history of a recent (<6 months) cardiovascular event or with symptomatic heart disease (New York Heart Association classes III and IV). The study protocol was approved by the Institutional Ethics Committee, KEM Hospital and Research Centre, Pune, and informed written consent was obtained from all the individuals after explaining the purpose and nature of the study. Medical history of all individuals was noted, and a standardized physical examination including body weight, height, waist : hip ratio, blood pressure and electrocardiogram was carried out for each of the subject.

Sample preparation

Blood samples were centrifuged at 4000 *g* for 10 min to separate the plasma. The buffy coat was removed, and the erythrocytes were washed thrice in cold saline (9.0 g/L NaCl) and hemolysed by adding ice-cold ultrapure water (MilliQ plus reagent grade; Millipore, Bedford, MA) to yield a 50% hemolysate. Aliquots of hemolysate were stored at −80 °C. Plasma glucose, total cholesterol, triglyceride, high-density lipoprotein cholesterol and creatinine concentrations were measured using standard kits on an autoanalyser (Hitachi 902, Japan). HbA_{1c} was measured by using a high-performance liquid chromatography cation exchange column on D10 HbA_{1C} analyser (Bio-Rad Laboratories, Hercules, CA). Plasma insulin was measured using insulin kit (Mercodia, Uppsala, Sweden). Insulin resistance (HOMA-IR) and β-cell function (HOMA-β) were calculated on the basis of the fasting insulin and glucose concentrations using online calculator for specific insulin assay (homeostatic model assessment) [17].

Assessment of oxidative stress parameters

A. *Antioxidant enzymes*: Antioxidant enzymes from the hemolysate were measured in the following manner:

A.1 *Catalase* (*CAT*) *activity* was determined using the method described by Aebi [18]. The decomposition rate of H_2O_2 by CAT was monitored at 240 nm. One unit of CAT is equal to 1 mmol of H_2O_2 decomposed per minute. The enzyme activity was expressed as unit per milligram protein.

A.2 *Glutathione peroxidase* (*GPx*) *activity* was measured using the method of Pagila and Valentine [19]. One unit

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of enzyme is defined as the amount of enzyme required to convert 1 μmole substrate to product in 1 min. The enzyme activity was expressed as unit per milligram protein.

A.3 *Glutathione reductase* (*GR*) *activity* was determined using the protocol of Goldberg [20]. One unit was defined as the amount of enzyme required to oxidize 1 μmole of NADPH per minute at 25 °C. The enzyme activity was expressed as unit per milligram protein.

A.4 *Superoxide dismutase* (*SOD*) *activity* was assayed according to the method of Beauchamp and Fridovich [21]. One unit of enzyme activity was defined as the amount of enzyme necessary for 50% inhibition in the reduction of nitroblue tetrazolium rate. The enzyme activity was expressed as unit per milligram protein.

B. *Antioxidant molecules*

B.1 *Reduced glutathione* (*GSH*) content from the erythrocyte hemolysate was estimated using 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) according to the method of Teixeria and Meneghini [22]. DTNB reacts with reduced GSH yielding yellow-coloured 2-nitro-5-thiobenzoate, which was read at 412 nm, and GSH was expressed as nanomoles per milliltre.

B.2 *Vitamin C* (*ascorbic acid*) from the plasma was measured spectrophotometrically at 520 nm using 2,4-dinitrophenylhydrazine (DNPH) according to the method of Shenkin [23]. After deproteinizing the plasma with trichloroacetic acid, 2,4-dinitrophenyl hydrazine/thiourea/copper was added, which results in the formation of dihydroascorbate products, which can be read at 520 nm and expressed as milligrams per decilitre.

B.3 *Uric acid* was estimated using Accurex kit (Accurex Biomedical Pvt. Ltd, India). Uricase converts uric acid into allantoin and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide oxidatively couples with phenolic chromogen to form a red-coloured compound, which was read at 510 nm and expressed as milligrams per decilitre.

B.4 *Bilirubin* was estimated using Accurex kit. Both conjugated bilirubin and unconjugated bilirubin react with diazotized sulfanic acid to form a pink-coloured chromogen, which was read at 546 nm and expressed as milligrams per decilitre.

C. *Oxidative damage markers*

C.1 *Protein carbonyl* concentration was estimated according to the method of Levine [24] using DNPH reagent. DNPH reacts with carbonyl groups forming stable hydrazone products, which was read at 365 nm. Results were expressed as nanomoles of protein carbonyls formed per milligram protein.

C.2 *Protein sulfhydryl* concentration was determined according to the method of Ellman [25] by using DTNB. Absorbance was measured at 412 nm against blank samples without DTNB and expressed as nanomoles per milligram protein.

C.3 *Protein concentration* in plasma and erythrocyte hemolysate was determined by the method of Lowry *et al*. [26].

C.4 *Lipid peroxides* were measured from the plasma in terms of nanomoles of malondialdehydes formed following the protocol of Heath and Packer [27]. After deproteinizing the plasma using trichloroacetic acid, thiobarbituric acid reagent was added, and these samples were boiled at 95 °C for 30 min and cooled immediately on ice. The pink colour developed due to thiobarbituric acid reactive substances (TBARs) was measured spectrophotometrically at 532 nm.

C.5 *8-hydroxy-2′-deoxyguanosine* (*8-OHdG*): DNA was isolated from whole blood by chloroform/isoamyl alcohol extraction method, and 8-OHdG concentration was determined by competitive enzyme-linked immunosorbent assay following the protocol of Modak *et al*. [28] and expressed as nanogram of 8-OHdG per 100 ng DNA.

Statistical analyses

Data are presented as mean ± standard deviation (SD). The baseline difference of oxidative stress parameters between diabetic patients and non-diabetic subjects was tested using *t*-test. Age and body size [weight, body mass index (BMI), and waist and hip circumference] in the two groups were significantly different, and therefore, the baseline difference of oxidative stress parameters in the two groups was further tested using multiple linear regression analysis, adjusted for gender, age and body size (BMI). Association of oxidative stress parameters with glycaemia and lipids was tested using multiple linear regression analysis (adjusted for gender, age and BMI). The strength is shown as standardized (std) β (SD change in outcome is per SD change in exposure). Longitudinal analysis was performed using repeated measures analysis of variance to test differences in serial measurements. We used generalized estimating equation to test the association of oxidative stress parameters with FPG [29].

We measured 12 different oxidative stress parameters that have different units and behave differently during the study period. To facilitate the interpretation of total oxidative

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stress in diabetic patients, we calculated oxidative stress score by computing gender-specific *Z*-scores of each parameter relative to the measurements in the control group. In the calculation of oxidative stress score, lower activities of CAT, GPx, GR and SOD, and lower concentrations of vitamin C, GSH and protein sulfhydryl contributed to higher oxidative stress score. On the other hand, higher concentrations of bilirubin, uric acid, protein carbonyl, TBARs and 8-OHdG contributed to higher oxidative stress score. This allows us to interpret and compare widely different measures using comparable and similar units. Association of oxidative stress score with fasting glucose, insulin, HOMA-IR and HOMA-β was tested using regression analysis. Statistical analysis was performed using statistical package R 2.9.2 and SPSS 16.0 (SPSS Inc., Chicago, USA).

Results

Demographic, anthropometric and biochemical parameters

We studied 54 T2D patients (28 men and 26 women) and 50 non-diabetic subjects (25 men and 25 women). Table 1 shows their demographic and anthropometric characteristics at baseline. Diabetic patients were older, more obese (BMI) and more centrally obese (waist circumference) compared with non-diabetic subjects ($p < 0.01$, all). Table 2 shows their biochemical characteristics at baseline and successive visits. In addition to higher FPG and HbA_{1C}, diabetic patients also had higher insulin, HOMA-IR, cholesterol and triglyceride concentrations at baseline ($p < 0.01$, all) but lower HOMA-β. Haemoglobin, high-density lipoprotein and creatinine concentrations were similar in the two groups ($p > 0.05$). Resting electrocardiogram was normal for all the diabetic patients and non-diabetic subjects. Creatinine concentrations significantly decreased in diabetic patients ($p < 0.001$) during the study; this was not associated with change in glucose concentrations.

Table 1. Demographic and anthropometric characteristics of study participants at baseline and follow-up; values are mean ± SD

	Controls	Diabetic patients	
	($n = 50$)	($n = 54$)	*p*-value
Gender (male/female)	25/25	28/26	—
Age (years)	33 ± 11	48 ± 10	<0.001
Height (cm)	156.8 ± 3.7	158.8 ± 7.4	0.08
Weight (kg)	58.8 ± 9.3	65.5 ± 12.5	0.02
Body mass index (kg/m^2)	23.9 ± 2.3	26.1 ± 3.9	0.02
Waist (cm)	84.2 ± 12.9	97.5 ± 9.8	<0.001
Hip (cm)	89.8 ± 12.9	103.0 ± 9.0	<0.001
Waist : hip ratio	0.93 ± 0.03	0.94 ± 0.03	0.60

Serial changes in glycaemia

Fasting plasma glucose concentrations showed minor and clinically non-significant changes in non-diabetic subjects over the 8 weeks of study period. After diagnosis, all diabetic patients were advised on diet and physical activity and received oral anti-diabetic treatment: 23 (42%) patients were treated with a combination of metformin and sulfonylurea (glimepiride or gliclazide), whereas 30 (55%) patients were treated with saxagliptin (dipeptidyl peptidase-4 inhibitor). Fifteen (28%) patients received statins. FPG and HbA_{1C} concentrations fell serially during the study period ($p < 0.01$, all).

Oxidative stress parameters

Oxidative stress parameters for non-diabetic subjects and diabetic patients for the three visits are summarized in Table 3. Compared with those in non-diabetic subjects, the antioxidant defence status was found to be impaired and oxidative markers elevated in the diabetic patients at the time of diagnosis. Of the antioxidant enzymes, activities of CAT, GPx and GR were significantly lower in diabetic patients ($p < 0.01$); however, the activity of SOD was comparable in the two groups. Of the antioxidant molecules, uric acid was significantly higher and GSH significantly lower in diabetic patients ($p < 0.01$), and concentrations of bilirubin and vitamin C were comparable in the two groups. Of the oxidative damage markers, concentrations of protein carbonyls and 8-OHdG were significantly higher in the diabetic patients whereas protein sulfhydryls were significantly lower ($p < 0.01$, all), and concentration of TBARs was comparable in the two groups. There was no difference in the oxidative stress parameters with respect to the anti-diabetic treatment.

Oxidative stress parameters were associated with gender, age and BMI (Supporting information Table S1). There was a significant difference in oxidative stress parameters between non-diabetic subjects and diabetic patients. Therefore in multiple linear regression analysis, we adjusted for these parameters. In the non-diabetic subjects, FPG was inversely associated with GR (std $\beta = -0.60$), SOD (std $\beta = -0.58$) and protein sulfhydryl (std $\beta = -0.46$) and directly with bilirubin (std $\beta = 0.46$) and TBARs (std $\beta = 0.85$) ($p < 0.01$, all). In the diabetic patients, FPG was inversely associated with all the antioxidant enzymes [CAT (std $\beta = -0.47$), GPx (std $\beta = -0.42$), GR (std $\beta = -0.55$) and SOD (std $\beta = -0.56$)], vitamin C (std $\beta = -0.36$), GSH (std $\beta = -0.36$) and protein sulfhydryl (std $\beta = -0.30$), whereas it was directly associated with bilirubin (std $\beta = 0.82$), uric acid (std $\beta = 0.44$), protein carbonyls (std $\beta = 0.30$), TBARs (std $\beta = 0.32$) and 8-OHdG (std $\beta = 0.40$) ($p < 0.01$, all).

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Table 2. Biochemical characteristics of study participants at baseline and at follow-up

	Non-diabetic subjects ($n = 50$)				Diabetic patients ($n = 54$)			
Parameters	Baseline	4 weeks	8 weeks	p-value	Baseline	4 weeks	8 weeks	p-value
Fasting glucose (mmol/L)	4.8 ± 0.4	5.0 ± 0.6	5.0 ± 0.4	0.03	10.7 ± 3.3	8.3 ± 2.2	7.5 ± 1.6	<0.001
HbA_{1C} (%)	5.5 ± 0.4	—	5.6 ± 0.6	0.10	9.8 ± 2.1	—	7.7 ± 1.0	<0.001
HbA_{1C} (mmol/mmol)	36.6 ± 5.8		38.5 ± 7.4		83.9 ± 23.0		61.1 ± 11.1	
Fasting insulin (pmol/L)	53.4 ± 24.0	61.2 ± 27.6	57.6 ± 28.8	0.09	69.0 ± 48.0	72.0 ± 45.6	73.8 ± 54.6	0.91
HOMA-IR	1.3 ± 0.6	1.5 ± 0.6	1.4 ± 0.7	0.06	2.1 ± 1.3	2.0 ± 1.3	2.0 ± 1.3	0.44
HOMA-β	118 ± 35	123 ± 39	116 ± 39	0.49	45 ± 32	63 ± 43	75 ± 52	<0.001
Cholesterol (mmol/L)	4.2 ± 0.9	4.4 ± 0.8	4.2 ± 0.8	0.64	5.0 ± 1.0	4.4 ± 0.8	4.3 ± 0.8	<0.001
Triglycerides (mmol/L)	1.0 ± 0.5	1.2 ± 0.8	1.2 ± 0.6	0.37	2.0 ± 1.3	1.6 ± 0.7	1.6 ± 1.2	0.10
HDL cholesterol (mmol/L)	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	0.93	1.0 ± 0.2	0.9 ± 0.2	0.9 ± 0.2	0.44
Creatinine (μmol/L)	78.6 ± 16.8	80.4 ± 14.1	83.1 ± 17.6	0.54	79.5 ± 13.2	74.2 ± 14.1	72.4 ± 12.3	<0.001

Values are mean ± SD. p-values refer to significance of difference in serial measurements.
HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β, homeostasis model assessment of β-cell function; HDL, high-density lipoprotein.

Table 3. Antioxidant defence status and oxidative damage markers in study participants at baseline and at follow-up

	Non-diabetic subjects ($n = 50$)				Diabetic patients ($n = 54$)			
	Baseline	4 weeks	8 weeks	p-value	Baseline	4 weeks	8 weeks	p-value
Antioxidant enzymes								
Catalase (U/mg protein)	1761 ± 293	1722 ± 284	1735 ± 245	0.04	1379 ± 205	1628 ± 310	1567 ± 259	<0.001
GPx (U/mg Protein)	0.19 ±0.03	0.19 ± 0.04	0.19 ± 0.04	0.23	0.07 ± 0.02	0.16 ± 0.07	0.18 ± 0.04	<0.001
GR (U/mg protein)	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.23	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.08
SOD (U/mg protein)	66 ± 11	66 ± 11	67 ± 10	0.48	58 ± 6	61 ± 9	61 ± 10	0.19
Antioxidant molecules								
Bilirubin (mg%)	0.14 ± 0.07	0.15 ± 0.07	0.15 ± 0.05	0.79	0.19 ± 0.08	0.14 ± 0.07	0.15 ± 0.06	<0.001
Uric acid (mg%)	4.10 ± 0.49	4.26 ± 1.07	4.24 ± 0.97	0.56	5.07 ± 1.29	4.07 ± 0.83	4.01 ± 0.67	<0.001
Vitamin C (mg%)	1.4 ± 0.5	1.5 ± 0.5	1.6 ± 0.4	0.01	1.1 ± 0.6	1.3 ± 0.6	1.1 ± 0.6	<0.001
GSH (nmole/mL)	865 ± 338	851 ± 302	826 ± 278	0.40	143 ± 122	198 ± 80	334 ± 132	<0.001
Oxidative damage markers								
Protein carbonyl (nmole/mg protein)	4.7 ± 1.6	5.1 ± 1.4	5.1 ± 1.5	0.08	8.9 ± 3.7	7.3 ± 2.5	6.5 ± 1.6	<0.001
Protein sulfhydryl (nmole/mg protein)	1.55 ± 0.40	1.58 ± 0.39	1.55 ± 0.39	0.51	0.82 ± 0.44	1.12 ±0.34	1.07 ± 0.35	<0.001
TBARs (nmole MDA/L)	2.28 ± 0.68	2.17 ± 0.67	2.24 ± 0.50	0.22	2.93 ± 1.30	2.38 ± 0.85	2.33 ± 0.94	<0.001
8-OHdG (ng/100 ng DNA)	0.8 ± 1.0	—	—	—	5.1 ± 7.0	—	—	—

Values are mean ± SD. p-values refer to significance of difference in serial measurements.
GPx, glutathione peroxidase; GR, glutathione reductase; SOD, superoxide dismutase; GSH, glutathione; TBARs, thiobarbituric acid reactive substances; MDA, malondialdehyde; 8-OHdG, 8-hydroxy-2′-deoxyguanosine.

Serial changes in oxidative stress parameters and its association with glycaemia (Table 3)

The majority of metabolic markers were stable in non-diabetic subjects over the study period. In diabetic patients, FPG concentration decreased (30%) during the 2 months of treatment ($p < 0.01$); however it did not fall to levels similar to that of non-diabetic subjects. Of the antioxidant enzymes, activities of CAT (14%) and GPx (142%) increased significantly ($p < 0.01$, all), whereas those of GR (13%) and SOD (4%) remained constant. Of the antioxidant molecules, bilirubin (16%) and uric acid (21%) decreased, whereas GSH (135%) increased significantly during the treatment ($p < 0.01$, all). Vitamin C concentration (22%) showed a significant increase by 4 weeks of treatment but fell to pre-treatment levels by 8 weeks. Of the oxidative stress markers, protein carbonyls (28%) and TBARs (22%) decreased, whereas protein sulfhydryl (29%) increased significantly during the treatment (p 0.01, all). The activity of GPx and concentrations of bilirubin, uric acid and TBARs were comparable with those in non-diabetic subjects after 8 weeks of treatment.

Serial changes in all the oxidative stress parameters were significantly associated with changes in the FPG concentration ($p < 0.01$, all), but there was no difference in the pattern of oxidative stress parameters by the class of anti-diabetic drugs.

Oxidative stress score, glucose, HOMA-IR and HOMA-β

Expectedly, total oxidative stress score was higher in diabetic patients compared with non-diabetic subjects at the beginning of the study and showed a progressive fall with control of hyperglycaemia (Figure 1). The change in glucose levels

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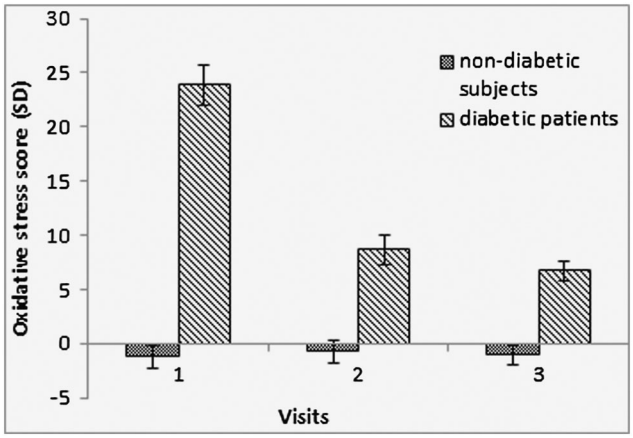

Figure 1. Oxidative stress score in non-diabetic subjects and diabetic patients. Oxidative stress score was calculated taking into account all the parameters measured to assess oxidative stress and compared between diabetic and non-diabetic subjects at baseline, visit 1(4 weeks) and visit 2 (8 weeks). Oxidative stress score was found to be significantly elevated in diabetic patients as compared with non-diabetic subjects and showed gradual reduction after 8 weeks of anti-diabetic treatment

was associated with the change in oxidative stress score (std $\beta = 0.74$, $p < 0.001$). There was 80% reduction in total oxidative stress score by 4 weeks, which thereafter remained stable by 8 weeks. The total score was still higher in diabetic patients at 8 weeks compared with that in non-diabetic subjects (Table 4). *Z*-scores of individual oxidative stress parameters is shown in Table S2.

In both non-diabetic subjects and diabetic patients, oxidative stress score was strongly associated with glucose levels (std $\beta = \sim 0.82$, $p < 0.001$, both) (Figure 2). In non-diabetic subjects, oxidative stress score was directly associated with HOMA-IR (std $\beta = 0.32$, $p < 0.01$) and inversely with HOMA-β (std $\beta = -0.17$, $p < 0.05$). In diabetic patients, there was no association between oxidative stress score and HOMA-IR, but there was a strong inverse association with HOMA-β (std $\beta = -0.56$, $p < 0.001$). Fall in oxidative stress score with treatment was associated with improvement in HOMA-β (std $\beta = -0.38$, $p < 0.01$).

Discussion

We undertook a comprehensive study of antioxidant enzymes, antioxidant molecules and oxidative stress markers in newly diagnosed diabetic patients and repeated the measurements serially for 8 weeks. A subgroup of 50 non-diabetic subjects served as controls. We observed a significantly impaired antioxidant defence and increased oxidative stress in newly diagnosed diabetic patients compared with non-diabetic subjects. This was clearly reflected in lower activities of antioxidant enzymes,

Table 4. Oxidative stress score at baseline and at follow-up; values are mean (95% CI)

	Non-diabetic subjects ($n = 50$)			Diabetic patients ($n = 54$)		
	Baseline	4 weeks	8 weeks	Baseline	4 weeks	8 weeks
Antioxidant enzyme score	1.0 (0.2, 1.8)	0.5 (−0.2, 1.4)	1.1 (0.3, 1.8)	−6.5 (−7.2, −5.8)	−1.9 (−3.1, −0.8)	−1.5 (−2.4, −0.7)
Antioxidant molecule score	−0.03 (−0.95, 0.87)	0.01 (−0.99, 1.03)	−0.01 (−0.90, 0.87)	5.98 (4.82, 7.13)	2.47 (1.54, 3.40)	2.64 (1.77, 3.51)
Oxidative damage score	−0.1 (−1.0, 0.8)	−0.2 (−0.9, 0.5)	0.1 (−0.6, 0.7)	11.2 (8.3, 14.0)	4.1 (2.5, 5.7)	2.90 (2.0, 3.7)
Total oxidative stress score	−1.2 (−3.4, 0.9)	−0.7 (−2.9, 1.4)	−1.1 (−2.9, 0.7)	23.8 (20.0, 27.6)	8.6 (5.8, 11.3)	7.1 (5.1, 9.1)

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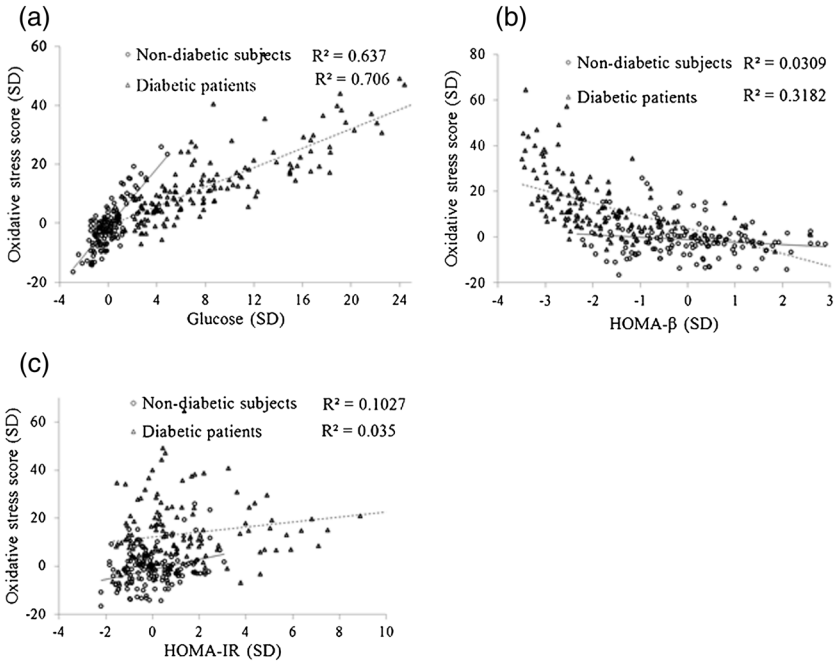

Figure 2. Association of oxidative stress score with glucose, HOMA-IR and HOMA-*β*. Multiple linear regression analysis was carried out to determine association between oxidative stress score and glucose (A), oxidative stress score and HOMA-IR (B) and oxidative stress score and HOMA-*β* (C). Oxidative stress score was found to be directly associated with glucose and inversely with HOMA-*β* and not with HOMA-IR. Regression line for non-diabetic subjects and diabetic patients are represented as — and ----, respectively

lower concentrations of antioxidants vitamin C and GSH, and elevated concentrations of a variety of oxidative stress markers despite elevation in endogenous antioxidants (bilirubin and uric acid). Serial measurements in non-diabetic subjects showed a stable status of oxidative stress parameters over 8 weeks. In diabetic patients, antioxidant defence status improved in response to anti-hyperglycaemic treatment. This was a consequence of elevated activity of antioxidant enzymes and concentrations of antioxidant molecules and decreased concentrations of oxidative stress markers. Of the oxidative stress parameters, concentrations of GPx, uric acid, bilirubin and TBARs became comparable with those in non-diabetic subjects. Level of glycaemia was a strong predictor of oxidative stress at baseline in diabetic patients, and fall in glucose levels over the study duration was a strong predictor of improved antioxidant status.

Several studies have reported a lowered status of antioxidant defence and elevated oxidative damage in diabetic patients with and without complications [30–33]. However, there are only a few reports on the antioxidative defence status of newly diagnosed diabetic patients [34–36] and even fewer studies wherein the effect of glycaemic control is serially measured [36]. There is an agreement in the literature that level of antioxidant defence is severely compromised and oxidative stress markers are elevated in diabetic patients. There is however no consensus on the behaviour of antioxidant enzymes in diabetic patients: some have shown an increase [8,10,33–37] while others a decrease [34,38–40] in the antioxidant enzyme activities. This discrepancy could be attributed to the difference in these studies with respect to type and duration of diabetes, glycaemic status, anti-diabetic treatment and also the methods used to measure different parameters. All the studies where association between oxidative stress parameters and glycaemia or diabetic complications is demonstrated are cross-sectional where it is difficult to assert causality. One study [36] demonstrated that oxidative stress was increased even in prediabetic state, that is, impaired glucose tolerance as reflected in high concentration of TBARs and low activity of SOD.

Intrinsic antioxidant defence machinery of a cell comprises several antioxidant enzymes and antioxidant molecules that scavenge ROS directly and/or indirectly, thereby protecting the cell from its deleterious effects. However, in diabetic patients, this defence machinery is severely impaired, thereby exacerbating the harmful effects of ROS. The possible mechanisms underlying this compromised antioxidant defence status are poorly understood. It is attributed to glycosylation and oxidation of proteins, lipids and nucleic acids. Disturbances in the redox buffer of a cell such as NADH/NAD and GSH/GSSG owing to the excess generation of free radicals also could contribute to the low defence status. Low concentration of GSH in turn fails to replenish antioxidants such as vitamins C and E [41] and further impairs the antioxidant defence mechanism. Another antioxidant, uric acid, at low concentrations constitutes over half the antioxidant

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capacity of blood plasma [42,43] but is known to act as a pro-oxidant at higher concentrations [44,45]. High levels of uric acid could therefore represent a compensatory response or a primary cause of oxidative stress. Excess ROS that escapes the antioxidant defence mechanism damages biomolecules such as proteins, lipids and DNA. ROS leads to the formation of carbonyl derivatives of proteins, degrades polyunsaturated fatty acids in all biological membranes to malondialdehyde and oxidizes DNA to 8-OHdG. Of these, only DNA can be repaired, whereas lipids and proteins are turned over. In the long run, this damage leads to disturbances in the integrity and physiological function of a cell, thereby contributing to various complications such as vascular damage in diabetic patients [46].

Improvement in the antioxidant defence status and oxidative stress markers in response to anti-hyperglycaemic treatment could be a direct consequence of the reduced ROS production. We found no difference between the diabetic patients receiving different anti-diabetic drugs with respect to the improvement in antioxidant defence status. Metformin [47] and gliclazide [48] have been reported to exhibit antioxidative property and are known to restore antioxidant function in T2D patients. There is no information available on specific antioxidant properties of saxagliptin.

Oxidative stress score, an indicator of total oxidative stress, showed strong correlation with glucose and HOMA-β, both in non-diabetic subjects and diabetic patients. Progressive decline of β-cell function in pre-diabetes is partially attributed to increasing 'hyperglycaemia' (glucotoxicity) [14,49]. β cells have a very poor antioxidant defence status [13] and fall prey easily to ROS assault. Improvement in HOMA-β in diabetic patients with improvement of hyperglycaemia is indicative of restoration of β-cell function as a consequence of reduced oxidative stress. In non-diabetic subjects, but not in diabetic patients, oxidative stress score had a weak association with HOMA-IR. Improvement in hyperglycaemia and reduction in oxidative stress score were not associated with HOMA-IR, suggesting irreversibility in short duration.

In summary, our findings provide a better understanding of oxidative stress in T2D. We found substantially elevated levels of oxidative stress at diagnosis of diabetes, which was associated with reduced β-cell function. Treatment of hyperglycaemia relieved a part of the oxidative stress irrespective of the type of anti-diabetic treatment and was associated with an improvement in β-cell function. It will be interesting to study the effect of stricter and longer glycaemic control regimen on oxidative stress and its potential benefits for β-cell function and other forms of tissue damage.

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Conflict of interest

The authors declare that there is no conflict of interest.

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