Chapter

Increase of Oxidants and Antioxidant Consumption in Patients with Type 2 Diabetes Mellitus in Peritoneal Dialysis

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Abstract

Oxidative stress (OS) is implicated as a unifying factor between chronic kidney diseases and cardiovascular diseases. The objective of the study was to compare the oxidant and antioxidant status in patients with PD according to the state of DM. Lipoperoxides (LPO), 8-isoprostanes (8-IP) and nitric oxide (NO) were determined as oxidants and the activity of superoxide dismutase (SOD) and total antioxidant capacity (TAC) as antioxidants in patients with DM and without DM (No-DM). We included 35 patients with DM, 42 No-DM patients and 10 healthy people as a control group (HC). Patients with DM were older ($p<0.0001$), had higher BMI ($p<0.0001$), high glucose levels ($p<0.0001$) and more hypertension ($p<0.0001$). It was found that LPO levels increased in patients with DM and No-DM vs. HC ($p<0.0001$). There was a decrease in the levels of 8-IP in DM and No-DM compared to HC ($p<0.0001$). The levels of NO in patients with DM and No-DM decreased significantly compared to the HC group with 197.97±34.20 μM ($p<0.0001$). The activity of the SOD enzyme in patients with DM and No-DM was found to be increased compared to the HC group ($p<0.0001$). The levels of TAC in HC were 2.62±0.17 mM and decreased in patients with DM and No-DM ($p<0.0001$).

Keywords: end-stage renal disease, chronic kidney disease, peritoneal dialysis, diabetes mellitus, oxidative stress, antioxidants

1. Introduction

Several traditional and nontraditional cardiovascular risk factors have been described in chronic kidney disease (CKD). Cardiovascular disease (CVD) still is the leading cause of death among end-stage renal disease (ESRD) patients. CVD and CKD are closely related to each other, and the disease of one of the organs causes dysfunction of the other by conditioning the failure of both organs [1]. CKD patients

generally have several traditional cardiovascular risk factors like diabetes mellitus (DM), dyslipidemia, and arterial hypertension. These conditions are associated with oxidative stress (OS), and these can trigger and accelerate the progression of renal injury [2]. OS is defined as the imbalance between oxidants and the antioxidant defenses of the body. Reactive oxygen species (ROS) are generated through enzymes such as nicotinamide phosphate adenine dinucleotide (NADPH) oxidase, which reduces oxygen to superoxide anion (O^{2-}). This anion is converted to hydrogen peroxide (H_2O_2) by the enzyme superoxide dismutase (SOD). The O^{2-} anion reacts with nitric oxide (NO) by producing peroxynitrite (nitrosative stress). The H_2O_2 reacts with intracellular iron to form the hydroxyl radical. In addition, H_2O_2 is catalyzed to hypochlorous acid in the presence of the chloride ion, by myeloperoxidase activity. Uremic toxins also participate by increasing ROS generation. The excessive production of ROS is able to oxidize lipids, proteins, and nucleic acids [3].

DM is a frequent cause of the need for renal replacement therapy (RRT) [4]. In Jalisco (Mexico), nearly half of the patients in dialysis are on peritoneal dialysis (PD), and almost half of these patients have DM [5]. In 2009, it was reported that diabetic nephropathy (DN) causes ~44% of all cases of ESRD in the United States [6]. DM contributes, in large part, to the high costs of health care and the increase in mortality from the increased incidence of DN that leads to ESRD [7]. The purpose of the study was to compare the oxidants and antioxidants state in patients with PD according to DM status.

2. Patients and methods

A single-center, analytical cross-sectional study was performed with ESRD patients on PD. Patients were eligible if they were 16 years old or older and were incident or prevalent in the population of the PD program and never have had a peritoneal equilibrium test (PET) performed before or if the last PET result was older than 1 year. We exclude patients with a current or a previous peritonitis episode in the last 6 months, patients with PD catheter dysfunction, and patients with a current infectious, inflammatory, and malignant process or impaired glycemic control (serum glucose > 200 mg/dL). We collected information about PD treatment and doses, residual diuresis, ultrafiltration, and the value of D/P creatinine (creatinine ratio in the dialysis fluid and plasma), reported at 4 h at the end of the PET. The type of peritoneal transport is determined by the result obtained, modality and the dialysis glucose solution concentration [8].

2.1 Oxidative stress markers

For measurement of OS markers, 10 mL of blood samples were drawn when PET blood samples were taken, 5 mL with 0.1% of ethylenediaminetetraacetic acid (EDTA) tube and other 5 mL in dry tube. The blood was immediately centrifuged at 10,000 rpm for 10 min at room temperature; supernatants were stored in aliquots at −80°C until their final processing. We included 10 mL of extra blood from 10 blood donors (healthy control) that was used to establish the normal value of the reagents.

2.1.1 Lipoperoxides (LPO)

The levels of LPO in plasma were measured through the FR22 assay kit (Oxford Biomedical Research Inc., Oxford, MI, USA®) according to the

manufacturer's instructions. The limit of detection for this test was 0.1 nmol/mL. The chromogenic reagent reacts with malondialdehyde (MDA) and 4-hydroxy-alkenals to form a stable chromophore. First, 140 μL of plasma with 455 μL of N-methyl-2-phenylindole in acetonitrile (Reagent 1) was diluted with ferric iron in methanol. Samples were agitated; after which 105 μL 37% HCl was added followed by incubation at 45°C for 60 min and centrifugation at 12,791 rpm for 10 min. Next, 150 μL of the supernatant was added, and absorbance was measured at 586 nm. The pattern curve with known concentrations of 1,1,3,3-tetramethoxypropane in Tris–HCl was used. The intra-assay CV was 8.5% [9].

2.1.2 8-Iso-prostaglandin $F_{2\alpha}$ 8-isoprostanes (8-IP)

The immunoassay reagent kit from Cayman Chemical Company® (Michigan, USA) was used according to the manufacturer's instructions. The limit of detection was 0.8 pg/mL. The 8-IP assay was based on the principle of competitive binding between samples 8-IP, 8-IP acetylcholinesterase (AChE) conjugate, and 8-IP tracer. Then, 50 μL of samples or standard was added to each well, and 50 μL of 8-IP AChE tracer was added to all wells except the total activity and blank wells; and 50 μL of 8-IP enzyme immunoassay antiserum was added to all wells except the total activity and blank wells. At once, 50 μL of 8-IP antiserum was added to all wells except total activity, non-specific binding, and blank wells. The plate was covered and incubated at 4 °C for 18 h and then washed 5 times with buffer. Absorbance was read at 420 nm. The intra-assay CV was 12.5% [10].

2.1.3 Nitric oxide (NO)

Prior to the determination of the NO levels, the serum samples were deproteinized by the addition of 6 mg of zinc sulfate to 400 μL of sample and vortexed for 1 minute and the samples were centrifuged at 10,000×*g* for 10 min at 4°C. For the determination of ON, the colorimetric method was used according to the kit (Nitric Oxide Assay Kit, NB98, Oxford Biomedical Research®). About 85 μL of the standard or sample was added to the wells of the plate, 10 μL of nitrate reductase was added to each well, and 10 μL of 2 mM NADH was added to the wells. The plate was stirred for 20 min at room temperature. Then 50 μL of dye 1 was added and stirred briefly and then 50 μL of dye 2, and again the samples were vortexed for 5 min at room temperature. Finally, the plate was read at 540 nm in a spectrophotometer within the first 20 minutes of completion of the procedure [11].

2.2 Antioxidants

2.2.1 Superoxide dismutase (SOD)

We followed the kit manufacturer's instructions (SOD No. 706002, Cayman Chemical Company®, USA) for the detection of O_2^- generated by the xanthine oxidase and hypoxanthine enzymes through the reaction of tetrazolium salts. We diluted the serum samples 1:5 in the sample buffer, 200 μL of the radicals' detector (1:400 dilution), and added 10 μL of the sample. After slow agitation, 20 μL of xanthine oxidase was then added to the wells. Then, the microplate was incubated for 20 minutes at room temperature. The absorbency was read at 440 wavelength of nm. The levels are reported in IU/mL [12].

2.2.2 Total antioxidant capacity (TAC)

The evaluations of TAC were made following the instructions of the kit manufacturer (Total Antioxidant Power Kit, No. TA02.090130, Oxford Biomedical Research®), to obtain the concentration in mM equivalents of uric acid. The detection limit was 0.075 mM. The samples and standards were diluted 1:40, and 200 μL was placed in each well. The plate was read at 450 nm as a reference value, 50 μL of copper solution was added, and the plate was incubated at room temperature for 3 minutes. Afterward, 50 μL of stop solution was added and the plate was read at 450 nm. The dilution factor was considered in the result. The intra-assay CV was 7.8% [13].

2.3 Statistical analysis

Normally distributed variables were presented as mean ± standard deviation (SD); skewed variables were exhibited as median with interquartile range (IQR). Categorical variables were expressed as frequency and percentage. All demographic and PD-related characteristics were compared between diabetics and nondiabetic patients using Chi^2, Student's *t* test, or Mann-Whitney U test accordingly to the type of data distribution. When significant differences in serum levels of oxidative stress markers were found between groups and were feasible, we conducted a multivariable analysis, to determine the interaction between DM and other factors associated with increased OS. The statistical analyses were performed using the IBM SPSS v.18 software (Chicago, IL, USA). For all the analysis, a $p \leq 0.05$ value was considered as statistically significant.

2.4 Ethics considerations

The scientific research study abides by the regulations of the internationally established guidelines of the Declaration of Helsinki 1964, revised in October 2013 at the World Medical Assembly. All procedures were performed according to regulations stipulated in the General Health Legal Guidelines for Healthcare Research in Mexico, 2nd Title, in Ethical Aspects for Research in Human Beings, Chapter 1, Article 17. The study was evaluated and approved by the Local Ethics and Research Committee at the Regional General Hospital No. 46, Mexican Institute of Social Security, Guadalajara, Jalisco, Mexico, with registration number R-2017-1303-117. All patients gave and signed the informed consent form in the presence of signed witnesses. Patients had the right to withdraw from the study at any time without representing harm to the patient-doctor relationship and without affecting their treatment. At all times, total confidentiality was maintained, and the patients were informed of the results throughout the study.

3. Results

Seventy-seven patients were included in the study, 35 with DM and 42 No-DM patients. The median age was 42 years. There was a greater prevalence of male-gendered patients, DM 62.86% and No-DM 76.19%. DM patients were older ($p < 0.0001$) and had higher body mass index ($p < 0.0001$), with significant increase in glucose ($p < 0.0001$) and more prevalence of arterial hypertension ($p < 0.0001$). A total of 63 patients used erythropoiesis-stimulating agents, with a mean dose of 140 ± 63.6 UI/kg/week, and a major dose was observed in No-DM patients. In **Table 1**, the demographic and biochemical results of the patients are shown. Significant increases in DM patients were found in triglyceride levels

	DM, n = 35	No-DM, n = 42	*p*
Gender n (%)			
Male	22 (62.86)	32 (76.19)	0.377
Female	13 (37.14)	10 (23.81)	1
Age, years	62 (54.5–66.5)	28 (25–37)	**<0.0001***
Weight, kg	73.16 ± 11.60	67.25 ± 12.52	**0.011****
Height, m	1.62 ± 0.09	1.66 ± 0.08	**0.046****
BMI, kg/m^2	27.80 ± 4.56	24.52 ± 4.29	**<0.0001****
Hypertension, n (%)	34 (97.14)	5 (11.90)	**<0.0001** [t]
Residual uresis, mL	500 (250–1000)	500 (57.5–1000)	0.85
Hemoglobin, g/dL	11.41 ± 1.80	10.83 ± 2.33	0.16
Urea, mg/dL	102.63 ± 30.95	124.39 ± 31.16	**<0.0001****
Creatinine, mg/dL	7.86 ± 3.07	13.89 ± 4–10	**<0.0001****
Glucose, mg/dL	131 (98–157)	95 (88.7–103)	**<0.0001***
Phosphorus, mg/dL	4.11 ± 1.42	5.63 ± 1.70	**<0.0001****
Calcium, mg/dL	8.76 ± 0.97	8.73 ± 0.92	0.35
Magnesium, mg/dL	2.13 ± 0.48	2.33 ± 0.41	**0.023****
Potassium, mEq/L	4.20 ± 0.73	4.47 ± 0.58	**0.032****
Sodium, mEq/L	138 (136–140)	140 (137–141)	0.06
Albumin, g/dL	3.17 ± 0.59	3.80 ± 0.53	**<0.0001****
Uric acid, mg/dL	6.22 ± 1.23	6.68 ± 1.39	0.08
Iron, mcg/dL	73.35 ± 26.34	77.49 ± 40.85	0.87
TSAT %	35.30 ± 17.86	32.5 ± 23.44	0.41
Cholesterol, mg/dL	171.51 ± 51.21	170.27 ± 43.38	0.91
HDL, mg/dL	41.55 ± 13.64	45.29 ± 15.34	0.28
LDL, mg/dL	93.12 ± 36.35	100.11 ± 35.46	0.45
Triglycerides, mg/dL	150 (98–219.6)	124 (88–158)	**0.046***
VLDL, mg/dL	30 (19.75–45.75)	24.5 (17.5–32.2)	**0.049***
HCO3, mEq/L	20.33 ± 3.27	21.57 ± 3.24	0.17
RCP, mg/L	3.31 (1.41–9.64)	1.98 (1–4.36)	**0.034***

Values are mean ± standard deviation (SD), percentage (%) or median (25–75th percentile), and [t]Chi^2 test
**Mann-Whitney U test; **Student's t test.*
DM, diabetes mellitus; No-DM, without DM; BMI, body mass index; TSAT, transferrin saturation; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; HCO_3, bicarbonate; RCP, reactive C protein.

Table 1.
Demographic and biochemical results in patients with ESRD in PD with and without diabetes mellitus

($p < 0.046$), very-low-density cholesterol (VLDL) ($p < 0.05$) and highly specific reactive C protein (RCP) ($p < 0.034$), which suggests systemic inflammation. However, phosphorus ($p < 0.0001$) and albumin ($p < 0.0001$) were found to be decreased. No-DM patients showed better biochemical results in urea ($p < 0.0001$), creatinine ($p < 0.0001$), magnesium ($p < 0.009$), and potassium ($p < 0.047$).

3.1 Dialysis characteristics

The patients had a median vintage on PD of 13 months (4–26), without differences between DM and No-DM. In addition, there were no differences in the

glucose concentration used in the dialysis solutions, but DM patients were less likely to be on automated peritoneal dialysis (APD) modality (p = 0.016), and the median dwell time was 16 h (QR 16–24). There were no differences in the D/P creatinine or the history of peritonitis between patients. The median residual diuresis was 500 mL/day (250–1000), and there were no difference in the volume of urine or the number of patients with significant residual diuresis. The peritoneal ultrafiltration and the total ultrafiltration were similar in both DM and No-DM patients (p = 0.54 and 0.72, respectively) and were within guidelines and recommendations.

3.2 Oxidants

3.2.1 Lipoperoxides, 8-iso-prostaglandin $F_{2\alpha}$ (8-IP), and nitric oxide

The levels of LPO (MDA and 4-hydroxy-alkenals) in the healthy controls were 3.05 ± 0.18 μM. However, LPO levels were found to be increased in DM patients, 136.95 ± 18.17 μM ($p < 0.0001$), and 194.18 ± 54.70 μM ($p < 0.0001$) in No-DM patients. No significant difference was found between patients with and without DM.

The serum levels of 8-IP in DM patients were found to be decreased 5.36 ± 0.80 pg/mL ($p < 0.0001$) and No-DM 9.99 ± 6.41 pg/mL ($p < 0.0001$) versus the levels that were obtained in healthy controls, 22.88 ± 3.80 pg/mL ($p < 0.0001$). No significant difference was found between patients with and without DM.

The levels of the NO products (nitrites/nitrates) in healthy controls were 197.97 ± 34.20 μM. The products of NO in DM patients were found to be decreased, 18.02 ± 3.41 μM ($p < 0.0001$), and No-DM, 12.96 ± 2.78 μM ($p < 0.0001$). There was no significant difference between patients with and without DM (**Table 2** and **Figure 1**).

3.3 Antioxidants

3.3.1 Superoxide dismutase and total antioxidant capacity

An increase in the serum activity of SOD was found in patients in PD with DM, 3.36 ± 0.28 U/mL, and No-DM, 3.28 ± 0.22 U/mL ($p < 0.0001$), versus the activity

	Healthy control	No-DM	p, MW-U	DM	p, MW-U
Antioxidants					
SOD, U/mL	0.23 ± 0.015	3.28 ± 0.22	**<0.0001**	3.36 ± 0.28	**<0.0001**
Total antioxidant capacity, mM	2.62 ± 0.17	0.69 ± 0.23	**<0.0001**	0.65 ± 0.031	**<0.0001**
Oxidants					
8-Isoprostane, pg/mL	22.88 ± 3.80	9.99 ± 6.41	**<0.0001**	5.36 ± 0.80	**<0.0001**
LPO, μM	3.05 ± 0.18	194.18 ± 54.70	**<0.0001**	136.95 ± 18.17	**<0.0001**
Nitric oxide, μM	197.97 ± 34.20	12.96 ± 2.78	**<0.0001**	18.02 ± 3.41	**<0.0001**

SOD, superoxide dismutase; LPO, lipoperoxides; No-DM, without diabetes mellitus; DM, diabetes mellitus; MW-U, Mann-Whitney U test.

Table 2.
Antioxidants and oxidants with and without diabetes mellitus vs. healthy controls.

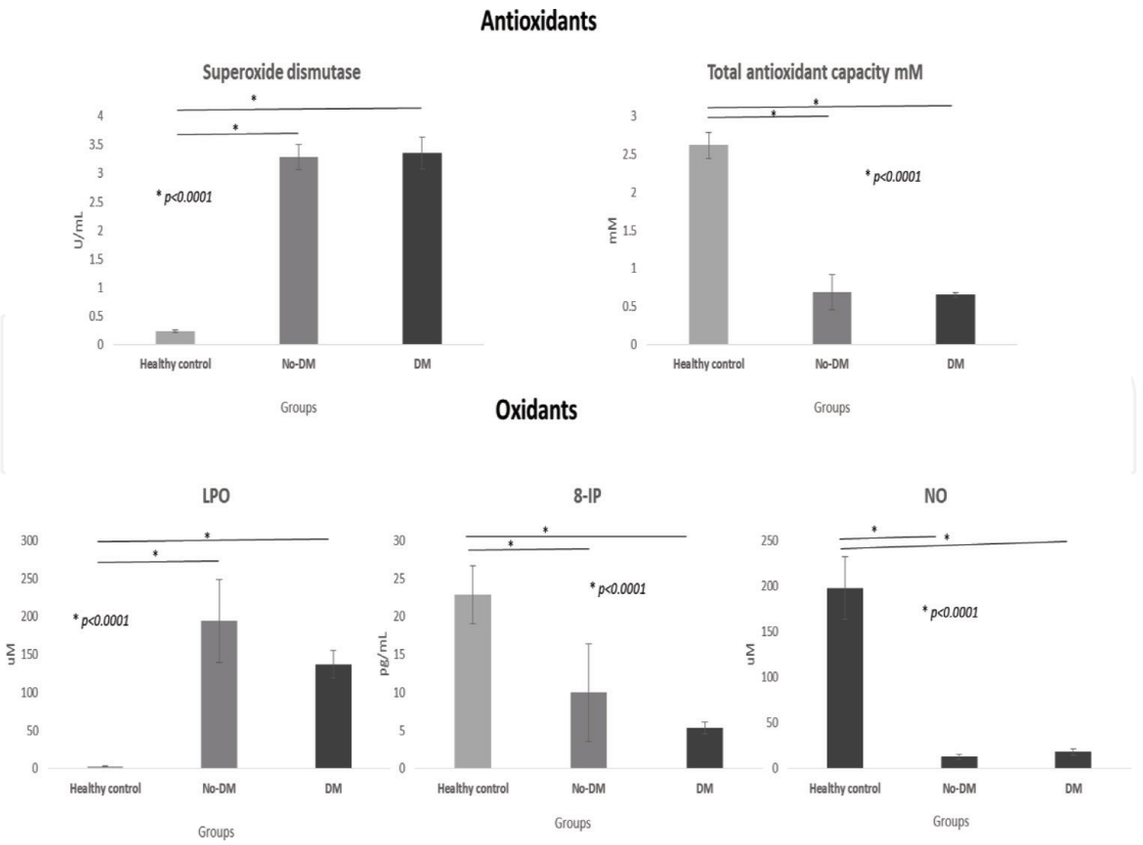

Figure 1.
Levels of antioxidant and oxidant in DM and No-DM patients.

of the enzyme in healthy controls, 0.23 ± 0.015 U/mL. There was no significant difference between patients with and without DM.

The serum levels found in healthy controls of TAC were 2.62 ± 0.17 mM; however, significantly decreased levels were found in DM patients, 0.65 ± 0.03 mM, and No-DM patients, 0.69 ± 0.23 mM ($p < 0.0001$). There was no significant difference between patients with and without DM (**Table 2** and **Figure 1**).

3.4 Correlations in all patients

When including the results of all patients, positive correlation was found between LPO with total cholesterol, LDL, triglycerides, and VLDL cholesterol. The 8-IP showed positive correlation with the reactive C protein. The NO products obtained a positive correlation with Hb, LDL, triglycerides, and VLDL cholesterol. The activity of SOD showed a positive correlation with creatinine, magnesium, and HDL cholesterol. Negative correlation was found between the products of NO and HDL cholesterol. SOD activity showed negative correlation with residual urine, Hb, triglycerides, and VLDL cholesterol (**Table 3**).

3.5 Correlations in no-DM patients

When including the results according to status in No-DM patients, there was a positive correlation between 8-IP with hemoglobin, between NO with triglycerides and LDL cholesterol, between SOD with creatinine and phosphorus, and between TAC with phosphorus and uric acid. There was negative correlation between LPO and body weight, NO and HDL cholesterol, SOD with residual diuresis, hemoglobin, triglycerides, and VLDL cholesterol (**Table 4**).

r^2	LPO	8-IP	NO	SOD	TAC
Weight	−0.12	0.05	0.02	0.06	0.03
Height	−0.10	−0.13	−0.008	−0.01	0.12
BMI	−0.05	0.15	0.04	0.08	−0.03
Residual urine	−0.007	0.04	0.061	**−0.31****	−0.09
Hemoglobin	0.07	0.09	**0.27***	**−0.38****	−0.05
Urea	−0.05	−0.01	**−0.26***	0.08	**0.36****
Creatinine	−0.14	−0.14	−0.16	**0.31****	**0.23***
Glucose	0.12	0.03	0.20	0.06	0.14
Phosphorus	−0.05	0.05	**−0.31****	0.17	**0.35****
Calcium	0.09	0.04	0.006	−0.21	0.03
Magnesium	−0.08	0.14	−0.13	**0.27***	0.07
Potassium	−0.10	0.13	−0.18	0.14	0.09
Sodium	0.16	−0.04	−0.01	−0.09	0.03
Albumin	0.06	−0.15	0.01	−0.08	0.15
Uric acid	−0.04	0.03	0.05	−0.06	**0.52****
Iron	−0.05	−0.05	−0.02	−0.04	0.07
TSAT	−0.19	0.42	−0.08	−0.14	−0.07
Cholesterol	**0.26***	0.02	0.15	−0.16	−0.08
HDL	−0.05	−0.01	**−0.44****	**0.29***	−0.21
LDL	**0.27***	0.007	**0.31***	−0.14	0.06
Triglycerides	**0.35****	0.04	**0.52****	**−0.49****	0.04
VLDL	**0.29***	0.07	**0.52****	**−0.44****	0.08
HCO_3	−0.07	−0.11	0.07	−0.03	0.03
RCP	−0.12	**0.37****	0.17	−0.19	0.08

*$p < 0.05$, **$p < 0.01$.
DM, diabetes mellitus; No-DM, without DM; BMI, body mass index; TSAT, transferrin saturation; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; HCO_3, bicarbonate; RCP, reactive C protein; LPO, lipoperoxides; 8-IP, 8-isoprostanes; NO, nitric oxide; SOD, superoxide dismutase; TAC, total antioxidant capacity.

Table 3.
Correlation between demographic, biochemical, and oxidative stress markers.

3.6 Correlations in DM patients

In patients with DM, positive correlation was found between LPO, calcium, cholesterol, and triglycerides; between 8-IP with calcium and RCP; between NO and VLDL cholesterol and LDL cholesterol and triglycerides; between SOD with creatinine, magnesium, and HDL cholesterol; and between TAC with urea, uric acid, VLDL cholesterol, and PCR. There was only a negative correlation between SOD with triglycerides and VLDL cholesterol and also between total cholesterol and HDL cholesterol (**Table 4**).

r^2	LPO	8-IP	NO	SOD	TAC
No-DM patients in PD					
Weight	**−0.34***	0.07	0.15	0.041	0.03
Height	−0.22	0.01	0.28	0.043	0.06
BMI	−0.26	0.06	0.03	0.005	0.01
Residual urine	0.06	0.20	0.08	**−0.38***	−0.05
Hemoglobin	−0.09	**0.32***	0.25	**−0.49****	−0.04
Urea	−0.16	0.02	−0.26	0.22	0.28
Creatinine	−0.15	−0.16	0.04	**0.50****	0.28
Glucose	−0.07	−0.06	0.13	0.19	0.10
Phosphorus	−0.10	−0.21	−0.27	**0.30***	**0.46****
Calcium	−0.13	0.06	−0.02	−0.18	−0.09
Magnesium	−0.004	0.04	0.08	0.24	−0.10
Potassium	−0.03	0.29	−0.13	0.22	0.23
Sodium	0.03	0.02	0.08	−0.02	−0.07
Albumin	0.08	0.18	0.27	−0.24	0.05
Uric acid	−0.02	0.05	0.10	−0.17	**0.51****
Iron	−0.08	−0.15	−0.02	−0.08	0.22
TSAT	−0.09	0.57	−0.06	−0.27	−0.16
Cholesterol	0.12	−0.05	−0.06	−0.07	−0.25
HDL	0.08	0.02	**−0.44****	0.19	−0.08
LDL	0.06	−0.01	0.17	−0.07	−0.04
Triglycerides	0.22	0.04	**0.46****	**−0.53****	−0.12
VLDL	0.21	0.05	**0.43****	**−0.48****	−0.11
HCO_3	−0.06	−0.08	0.14	−0.05	0.41*
PCR	−0.15	0.27	0.23	−0.11	−0.15
Diabetic patients in PD					
Weight	0.14	−0.05	−0.27	0.03	0.12
Height	0.05	−0.22	−0.21	−0.06	0.08
BMI	0.06	0.12	−0.15	0.10	0.05
Residual urine	−0.16	−0.16	−0.02	−0.21	−0.14
Hemoglobin	0.28	−0.21	0.23	−0.25	−0.02
Urea	0.13	0.03	−0.03	−0.03	**0.36***
Creatinine	−0.09	0.09	−0.17	**0.39***	0.17
Glucose	0.23	−0.01	0.20	−0.05	0.27
Phosphorus	0.12	**0.45****	−0.17	0.07	0.19
Calcium	**0.39***	0.04	0.11	−0.23	0.14
Magnesium	−0.07	0.34	−0.25	**0.35***	0.14
Potassium	−0.19	0.05	−0.16	0.06	−0.09
Sodium	0.28	0.002	−0.06	−0.13	0.10
Albumin	0.26	−0.32	−0.02	0.07	0.13
Uric acid	0.01	0.01	0.16	0.04	**0.53****

r^2	LPO	8-IP	NO	SOD	TAC
Iron	0.003	0.05	−0.007	0.05	−0.10
TSAT	−0.65	0.21	−0.14	0.09	−0.06
Cholesterol	**0.42****	0.08	**0.42***	−0.24	0.09
HDL	−0.21	−0.03	−0.33	**0.39***	**−0.38***
LDL	**0.55****	0.05	**0.52****	−0.24	0.19
Triglycerides	**0.46****	0.003	**0.54****	**−0.46****	0.32
VLDL	0.34	0.04	**0.57****	**−0.41***	**0.35***
HCO_3	−0.03	−0.14	0.09	−0.03	−0.20
RCP	−0.03	**0.50***	0.09	−0.38	**0.44***

$^*p < 0.05$, $^{**}p < 0.01$.
DM, diabetes mellitus; No-DM, without DM; BMI, body mass index; TSAT, transferrin saturation; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; HCO_3, bicarbonate; RCP, reactive C protein; LPO, lipoperoxides; 8-IP, 8-isoprostanes; NO, nitric oxide; SOD, superoxide dismutase; TAC, total antioxidant capacity.

Table 4.
Correlation between demographic, bioquimical, and oxidative stress markers.

4. Discussion

In this cross-sectional study, we compared levels of oxidative stress markers on PD patients with and without diabetes. The DM patients were older, had higher BMI, had more prevalence of hypertension, and had higher triglycerides and VLDL levels that corresponded to the classic metabolic syndrome [14]. The prevalence of metabolic syndrome in patients with ESRD on PD is quite frequent, and its presence is reported in ~40–60% of patients with DN depending on the population studied [15].

DM is the main cause of ESRD and represents 58% of incident cases [16]. Several interventions have been suggested for the prevention and control of DM. A directed selection study is followed by a randomized controlled trial by groups, with randomization where participants were at risk for DM. The subjects received standard care or intervention. The intervention consisted of a group structured education program of 6 h with an annual update and regular telephone contact with follow-up for 3 years. About 29.1% attended all the sessions; 22.6% did not attend the education. The authors found a 26% reduction in the risk of type 2 DM of those who received the intervention compared with the standard care. They found no statistical significance, which suggests that the effectiveness of all interventions is not promising [17].

When the patient already has DM, a variety of risk factors that promote the development and progression of DN have been reported: persistent high glucose levels, time of duration of DM, arterial hypertension, obesity, endothelial dysfunction, and dyslipidemia. Most of these risk factors are modifiable through antidiabetic, antihypertensive, lipid-lowering, and primarily lifestyle change [18]. It has been previously reported that patients with ESRD and DM who require dialysis have higher rates of various morbidities and worse clinical outcomes compared to non-DM patients. It has also been shown that glycemic decontrol is associated with more micro- and macrovascular complications, in addition to higher mortality [19].

In the present study, a significant increase in LPO was found in DM and No-DM patients undergoing PD *vs*. the levels found in healthy controls. It has been shown that patients in PD manifest excessive OS compared to healthy controls. OS in PD

is closely related to chronic inflammation, atherogenesis, peritoneal fibrosis, and loss of residual renal function. The unfavorable serum lipid profile and the chronic exposure of peritoneal cells to super-physiological levels of glucose in patients undergoing PD could increase glycosylation and lipid oxidation, favoring the increase of LPO and consequently greater OS [20]. When there is greater permeability and biocompatibility of the peritoneal membrane, with better preserved residual renal function, it can be assumed that patients could have a lower oxidative load [21, 22]; however, reports in the literature indicate that the oxidative metabolism of peripheral and peritoneal phagocytes is activated during PD with conventional dialysate, by the products of glucose degradation, by low pH, and by high osmolarity [23]. The bio-incompatibility of DP solutions seems to play a central role in increasing ROS production [24]. Previously increased OS in patients with ESRD in RRT has been reported before undergoing kidney transplantation [25].

In the results obtained in the patients undergoing PD with DM and No-DM, the 8-IP were significantly diminished versus the healthy controls; possibly, the clearance of this marker by PD participates in the decrease of serum levels. Previous reports have shown that the residual glomerular filtration rate is independently associated with the levels of advanced glycation end products in plasma effluents and peritoneum [26]. It has been previously reported that DM is associated with increased lipid peroxidation and persistent platelet activation with increased in vivo formation of F2-isoprostane 8-iso-prostaglandin (PG) F2alfa (bioactive product of arachidonic acid peroxidation). 8-IP improves their levels in presence of DM by contributing to platelet activation related to altered glycemic control and increased lipid peroxidation by providing an important biochemical link between altered glycemic control and persistent platelet activation [27]. However, in our study this marker was found to be significantly decreased in serum in both DM and No-DM patients in inverse relation to published reports where they found increased levels in urine [18]. Kant et al. mentioned that when lipid peroxidation products were elevated in the prediabetic stage, the determination of this marker is useful in the detection of patients at risk of type 2 DM [28]. In this study, the 8-IP in residual urine was not measured.

In patients with DM and No-DM, we found significantly decreased serum levels of NO. NO is a potent biological vasodilator produced by the vascular endothelium from L-arginine. NO is synthesized by endothelial NO synthase (eNOS). Vascular NO deficiency may be involved in accelerated atherosclerosis and the dramatic cardiovascular mortality observed in patients with CKD [29]. Vascular changes can induce OS and inflammation, favoring the probability of morbidity and mortality by aggravating CVD [30]. In CKD, endothelial dysfunction is characterized by the altered capacity of the vascular endothelium to stimulate vasodilation. NO plays a key role in the development of atherosclerosis in this pathology. The decrease in the bioavailability of NO is a key factor of endothelial dysfunction. In addition, NO plays an important role in the protection of the vascular wall because it induces its own metabolic products [31].

In the present study, a significant increase in the activity of the SOD enzyme was found in DM and No-DM patients undergoing PD. The accumulated scientific evidence suggests that the main antioxidant systems are impaired in patients on PD [32]; possibly the previous finding could be explained in an attempt to compensate the oxidative state that characterized the patients included in the study. Antioxidants can be divided into intracellular and extracellular antioxidants. The intracellular enzymatic antioxidants are SOD, catalase, and glutathione peroxidase, which convert substrates (anion radicals O^{2-} and H_2O_2) into less reactive forms. The first line of defense against free radicals is SOD. Free radicals are the source of lipid peroxidation derived from oxygen, and the function of SOD is to catalyze the conversion of O^{2-} radicals into H_2O_2. Therefore, if the activity of SOD in PD had been found to be decreased, it would suggest accumulation of the O^{2-} radical

anion responsible for the increase in lipid peroxidation [21]; however, in the patients included in the study, SOD was found to be significantly increased and TAC decreased in patients undergoing PD with DM and No-DM, which could suggest that peritoneal replacements could purge the systemic buffering levels of the TAC. In a recently reported study, the authors underwent to hemodialysis session in patients with ESRD; they found a significant reduction in TAC according to our findings [33]. The depletion of TAC found in patients undergoing PD is contrary to that reported by other authors in relation to increased levels of these systemic buffers in patients undergoing hemodialysis. [34]. The concentrations of bilirubin, uric acid, and plasma albumin are the main defense in the extracellular fluids generated during the normal metabolism or are ingested by the consumption of dietary products rich in antioxidants [35]. These extracellular antioxidants prevent the reaction of free radicals by sequestering transition metal ions by plasma chelation [36]; the TAC is able to determine these extracellular antioxidants. In the present study, TAC was found to be significantly consumed in all patients included.

The addition of exogenous antioxidants to the management of patients who are in PD is an incomplete and little studied subject; however, this topic is interesting that is well worth considering in these patients. In a report of the literature, the authors studied the N-acetylcysteine (NAC) which is considered as a potential antioxidant with anti-inflammatory effects for dialysis patients. Vitamin C could play an important role in helping PD patients to use iron for erythropoiesis and achieve better hemoglobin response during the treatment of anemia [37].

5. Conclusions

When comparing patients in PD according to the presence or absence of DM, we found imbalance of markers of oxidative stress characterized by increased LPO products, serum decrease of 8-PI, dysregulation of the antioxidant defense system with significant decrease in TAC, and increase in SOD possibly in an attempt to compensate for the state of oxidative stress. In this population, increased levels of triglycerides and VLDL were observed, which favors the appearance of accelerated atherosclerosis with an increase in arterial stiffness, as well as a decrease in NO levels, which favors secondary endothelial dysfunction. These factors contribute to the increase of CVD in PD patients. Supplementation with external antioxidants could be an emerging strategy to counteract OS with the potential to preserve peritoneal function.

Conflict of interests

There is no conflict of interest.

Abbreviations

OS	oxidative stress
CKD	chronic kidney diseases
PD	peritoneal dialysis
DM	diabetes mellitus
LPO	lipoperoxides
8-IP	8-isoprostanes
NO	nitric oxide
SOD	superoxide dismutase

TAC	total antioxidant capacity
No-DM	without diabetes mellitus
HC	healthy control
CVD	cardiovascular disease
ESRD	end-stage renal disease
ROS	reactive oxygen species
NADPH	nicotinamide phosphate adenine dinucleotide
D/P	creatinine ratio in the dialysis fluid and plasma (reported at 4 h at the end of the peritoneal equilibrium test)
O^{2-}	superoxide anion
H_2O_2	hydrogen peroxide
RRT	renal replacement therapy
DN	diabetic nephropathy
PET	peritoneal equilibrium test
EDTA	ethylenediaminetetraacetic
SD	standard deviation
IQR	interquartile range
TC	total cholesterol
LDL	low-density cholesterol
HDL	high-density cholesterol
VLDL	very-low-density cholesterol
RPC	reactive protein C
Hb	hemoglobin

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