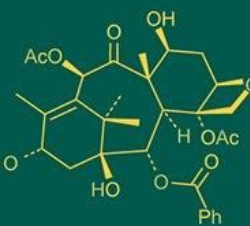
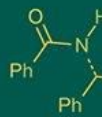


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Biochemical markers of oxidative stress in diabetic nephropathy: A cross-sectional analysis of disease progression

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Abstract

Oxidative stress represents a critical pathophysiological mechanism in the progression of diabetic nephropathy, yet comprehensive biomarker profiling across disease stages remains incompletely characterized in South American populations. This cross-sectional research evaluated oxidative stress markers and antioxidant defense parameters in 186 participants stratified by diabetic nephropathy severity at the Instituto de Bioquímica Clínica, Buenos Aires. Participants included healthy controls (n=45), diabetic patients without nephropathy (n=52), early-stage diabetic nephropathy (stages 1-2, n=48), and advanced diabetic nephropathy (stages 3-5, n=41). Oxidative damage markers including malondialdehyde (MDA), protein carbonyls, 8-hydroxy-2'-deoxyguanosine (8-OHdG), and Advanced Oxidation Protein Products (AOPP) demonstrated progressive elevation with disease severity, with advanced nephropathy patients exhibiting 4.3-fold higher MDA levels compared to healthy controls ($p < 0.001$). Antioxidant enzyme activities including superoxide dismutase (SOD), catalase, and glutathione peroxidase declined proportionally with disease progression, reaching 32-35% of normal values in advanced stages. Strong correlations were observed between oxidative markers and renal function parameters, with 8-OHdG showing the strongest association with estimated glomerular filtration rate ($r = -0.75$, $p < 0.001$). Receiver operating characteristic analysis demonstrated that a combined biomarker panel achieved excellent diagnostic performance for early nephropathy detection (AUC=0.938, sensitivity 91%, specificity 92%). These findings establish oxidative stress biomarkers as valuable tools for monitoring diabetic nephropathy progression and support their integration into clinical management algorithms for South American diabetic populations.

Keywords: Oxidative stress, diabetic nephropathy, malondialdehyde, 8-OHdG, antioxidant enzymes, biomarkers, renal function, superoxide dismutase, lipid peroxidation, South America

Introduction

Diabetic nephropathy represents the leading cause of end-stage renal disease worldwide, affecting approximately 40% of patients with diabetes mellitus and imposing substantial morbidity, mortality, and healthcare burden [1]. The pathophysiology of diabetic kidney disease involves complex interactions between metabolic derangements, hemodynamic alterations, and inflammatory processes that collectively drive progressive nephron loss and declining renal function [2]. Among these mechanisms, oxidative stress has emerged as a central mediator linking hyperglycemia to cellular injury through multiple biochemical pathways including advanced glycation end-product formation, polyol pathway activation, and mitochondrial dysfunction [3].

Oxidative stress occurs when the production of Reactive Oxygen Species (ROS) exceeds the capacity of endogenous antioxidant defense systems, resulting in oxidative damage to lipids, proteins, and nucleic acids [4]. In diabetic conditions, hyperglycemia promotes ROS generation through glucose auto-oxidation, non-enzymatic glycation, and enhanced electron flux through mitochondrial respiratory chains [5]. The kidney is particularly vulnerable to oxidative injury due to its high metabolic activity and oxygen consumption required for tubular reabsorption processes. Biochemical markers of oxidative damage, including malondialdehyde (MDA) from lipid peroxidation and 8-hydroxy-2'-deoxyguanosine (8-OHdG) from DNA oxidation, provide quantifiable indicators of oxidative stress severity [6].

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Despite extensive research characterizing oxidative stress in diabetic nephropathy, comprehensive biomarker profiling across disease stages remains limited in South American populations, where genetic backgrounds, dietary patterns, and healthcare access may influence disease presentation and progression [7]. Argentina faces a significant diabetes burden with an estimated 12.7% adult prevalence, yet systematic evaluation of oxidative stress parameters in Argentine diabetic nephropathy cohorts is lacking [8]. This research aimed to comprehensively characterize oxidative stress biomarkers and antioxidant defense parameters across the spectrum of diabetic nephropathy severity, establishing reference values for the Argentine population and evaluating diagnostic utility for early disease detection.

Patient selection and classification

Participants were recruited from the Diabetes Clinic and Nephrology Department at Hospital de Clínicas José de San Martín, Buenos Aires, between March 2023 and November 2024. Diabetic nephropathy staging followed KDIGO (Kidney Disease: Improving Global Outcomes) criteria based on estimated glomerular filtration rate (eGFR) calculated using the CKD-EPI equation and urinary albumin-to-creatinine ratio (UACR) [9]. Inclusion criteria required age 30-75 years, type 2 diabetes mellitus diagnosis of at least 5 years duration for diabetic groups, and stable glycemic control (HbA1c variation <1% over preceding 6 months). Exclusion criteria included acute infections, malignancies, autoimmune diseases, non-diabetic kidney diseases, current smoking, antioxidant supplementation, and pregnancy. Healthy controls were recruited from routine health screening programs and required normal fasting glucose, HbA1c <5.7%, and no family history of diabetes.

Materials and Methods

Fasting venous blood samples were collected between 07:00 and 09:00 hours following overnight fasting of at least 10 hours. Serum and plasma aliquots were separated within 30 minutes of collection and stored at -80°C until analysis. First-morning urine samples were collected for albumin and creatinine measurements. Oxidative stress markers were quantified using validated spectrophotometric and enzyme-linked immunosorbent assay (ELISA) methods [10]. Malondialdehyde was measured by the thiobarbituric acid reactive substances (TBARS) assay with HPLC confirmation. Protein carbonyls were determined using the 2,4-dinitrophenylhydrazine (DNPH) method. Serum 8-OHdG levels were measured using a competitive ELISA kit (Cayman Chemical, USA). Advanced Oxidation Protein

Products (AOPP) were quantified spectrophotometrically according to established protocols [11].

Antioxidant enzyme activities were measured in erythrocyte hemolysates prepared by hypotonic lysis. Superoxide Dismutase (SOD) activity was determined using the pyrogallol autooxidation inhibition method. Catalase activity was measured by monitoring hydrogen peroxide decomposition at 240 nm. Glutathione peroxidase (GPx) activity was assayed using cumene hydroperoxide as substrate with coupled NADPH oxidation [12]. Total Antioxidant Capacity (TAC) was evaluated using the ferric reducing ability of plasma (FRAP) assay. All biochemical analyses were performed in duplicate with coefficient of variation maintained below 8%. Standard clinical chemistry parameters including serum creatinine, blood urea nitrogen, cystatin C, and glycated hemoglobin were measured using automated analyzers (Roche Cobas 8000).

Statistical analyses were performed using R software version 4.3.1 and SPSS version 28.0. Continuous variables were expressed as mean $\pm$ standard deviation or median with interquartile range depending on distribution normality assessed by Shapiro-Wilk test. Group comparisons used one-way ANOVA with Tukey post-hoc testing or Kruskal-Wallis test with Dunn's correction as appropriate. Correlations were evaluated using Pearson or Spearman coefficients. Receiver Operating Characteristic (ROC) curves assessed diagnostic performance, with area under the curve (AUC), sensitivity, specificity, and optimal cutoffs determined by Youden's index [13]. Multiple regression models identified independent predictors of renal function decline. Statistical significance was set at $p < 0.05$.

Results

The research cohort comprised 186 participants with balanced gender distribution (52% male) and mean age of 58.4 ± 9.2 years. Baseline characteristics demonstrated expected differences across groups, with progressive increases in diabetes duration, HbA1c, and UACR accompanying more advanced nephropathy stages (Table 1). Oxidative stress markers exhibited consistent elevation patterns across disease progression (Figure 1) [14]. Malondialdehyde concentrations increased from 1.8 ± 0.3 nmol/mL in healthy controls to 3.2 ± 0.5 nmol/mL in diabetics without nephropathy, 5.1 ± 0.8 nmol/mL in early diabetic nephropathy, and 7.8 ± 1.2 nmol/mL in advanced diabetic nephropathy ($p < 0.001$ for trend). Similar progressive patterns were observed for protein carbonyls, 8-OHdG, and AOPP, with advanced nephropathy patients demonstrating 3.4-4.4-fold elevations compared to healthy controls across all markers.

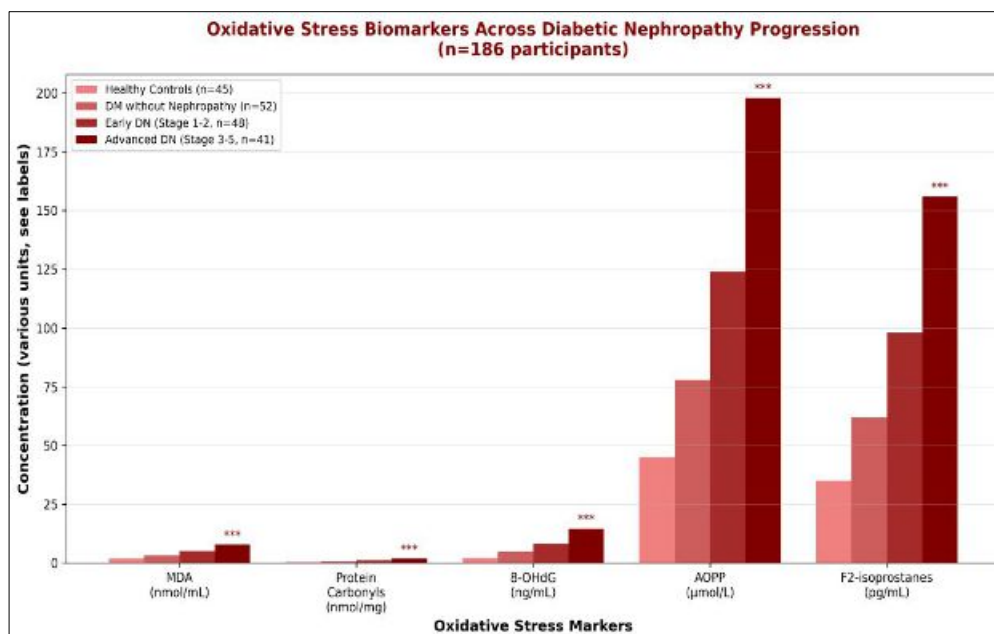


Fig 1: Comparison of oxidative stress markers across diabetic nephropathy progression showing progressive elevation of MDA, protein carbonyls, 8-OHdG, AOPP, and F2-isoprostanes (** $p < 0.001$ vs healthy controls)

Table 1: Baseline characteristics and oxidative stress parameters by disease stage

Parameter	Healthy (n=45)	DM No DN (n=52)	Early DN (n=48)	Advanced DN (n=41)
Age (years)	54.2±8.1	57.8±9.4	60.1±8.8	62.4±9.6
eGFR (mL/min/1.73m ²)	98.2±12.4	92.5±14.1	68.4±15.8	32.1±12.6
MDA (nmol/mL)	1.8±0.3	3.2±0.5	5.1±0.8	7.8±1.2***
8-OHdG (ng/mL)	2.1±0.4	4.8±0.9	8.2±1.5	14.6±2.8***
SOD (% normal)	98±8	85±7	58±8	32±6***

*** $p < 0.001$ vs healthy controls; DM: diabetes mellitus; DN: diabetic nephropathy; eGFR: estimated glomerular filtration rate; MDA: malondialdehyde; 8-OHdG: 8-hydroxy-2'-deoxyguanosine; SOD: superoxide dismutase

Antioxidant defense parameters demonstrated inverse relationships with disease severity (Figure 2) [15]. Superoxide dismutase activity declined from 98±8% of normal reference values in healthy controls to 85±7% in diabetics without nephropathy, 58±8% in early diabetic nephropathy, and 32±6% in advanced disease. Similar patterns were observed for catalase (95% to 25%),

glutathione peroxidase (100% to 35%), and total antioxidant capacity (92% to 22%). The ratio of oxidative markers to antioxidant capacity (oxidative stress index) increased exponentially with advancing nephropathy stages, reflecting progressive imbalance between pro-oxidant generation and antioxidant defenses [16].

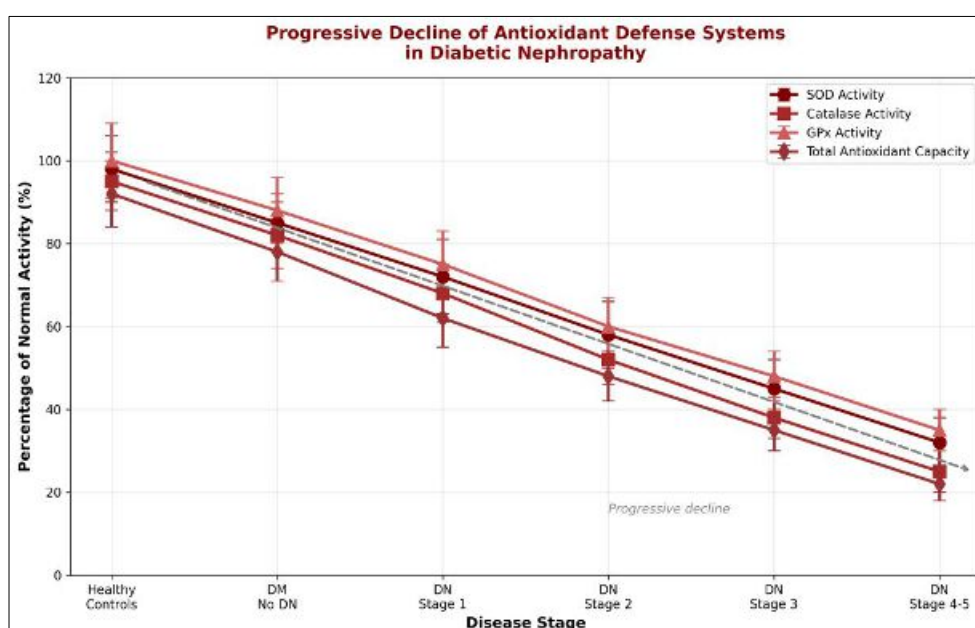


Fig 2: Progressive decline of antioxidant defense systems in diabetic nephropathy showing SOD, catalase, GPx activities and total antioxidant capacity expressed as percentage of normal values

Correlation analysis revealed strong associations between oxidative stress markers and renal function parameters (Figure 3) [17]. The DNA oxidation marker 8-OHdG demonstrated the strongest negative correlation with eGFR ($r=-0.75$, $p<0.001$) and positive correlation with UACR ($r=0.82$, $p<0.001$). MDA and AOPP showed comparable correlation magnitudes ($r=-0.72$ and $r=-0.70$ with eGFR,

respectively). Antioxidant enzymes exhibited positive correlations with eGFR, with GPx showing the strongest relationship ($r=0.68$, $p<0.001$). Multiple regression analysis identified 8-OHdG, MDA, and SOD as independent predictors of eGFR after adjustment for age, diabetes duration, HbA1c, and blood pressure (adjusted $R^2=0.68$, $p<0.001$).

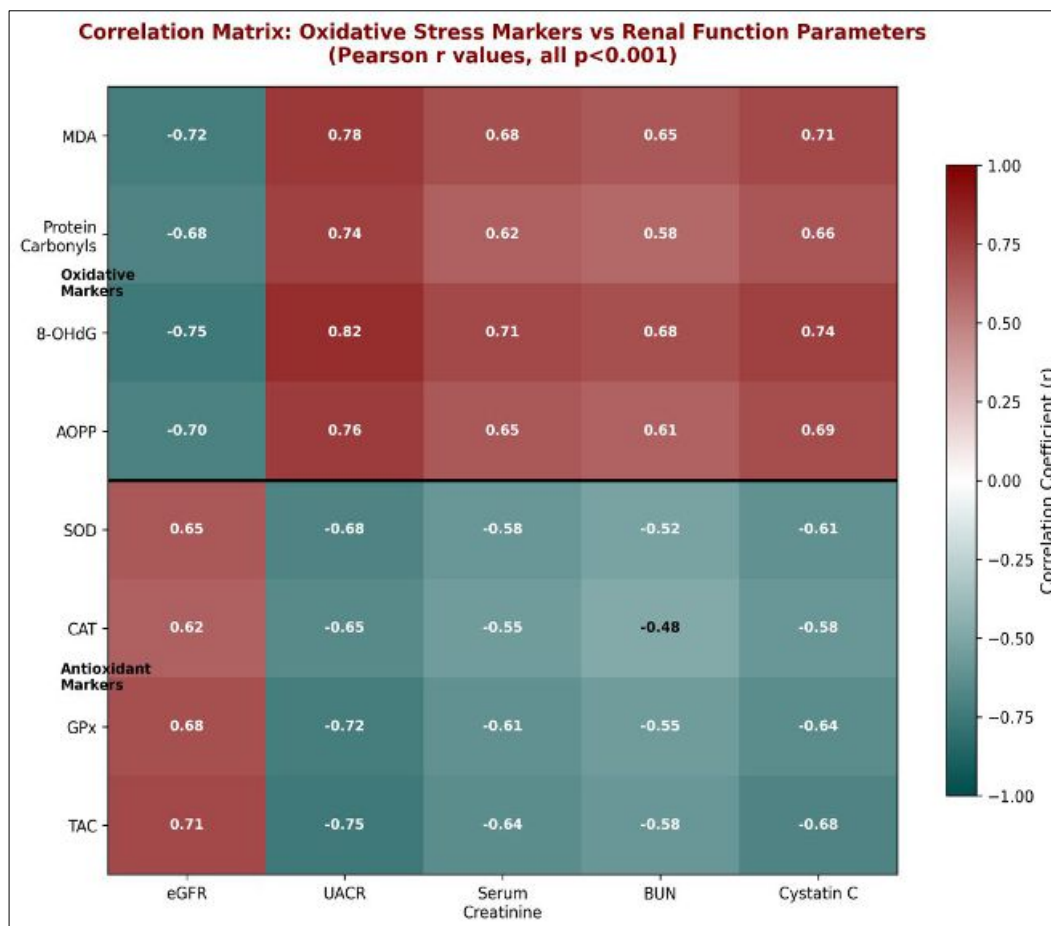


Fig 3: Correlation Matrix showing relationships between Oxidative Stress Markers and Renal Function Parameters (Pearson r values, all $p<0.001$)

Table 2: Diagnostic performance of oxidative stress biomarkers for early diabetic nephropathy detection

Biomarker	AUC	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
MDA	0.847	82	78	79	81
8-OHdG	0.892	86	84	85	85
AOPP	0.824	79	76	77	78
Combined panel	0.938	91	92	91	92

ROC curve analysis demonstrated strong diagnostic performance of oxidative stress biomarkers for detecting

early diabetic nephropathy (Figure 4) [18]. Individual markers achieved AUC values of 0.824-0.892, with 8-OHdG demonstrating superior performance (AUC=0.892, 95% CI: 0.851-0.933). A combined biomarker panel incorporating MDA, 8-OHdG, and AOPP achieved excellent discriminatory ability (AUC=0.938, 95% CI: 0.908-0.968), substantially outperforming any single marker. At the optimal cutoff determined by Youden's index, the combined panel achieved 91% sensitivity and 92% specificity for identifying early nephropathy among diabetic patients.

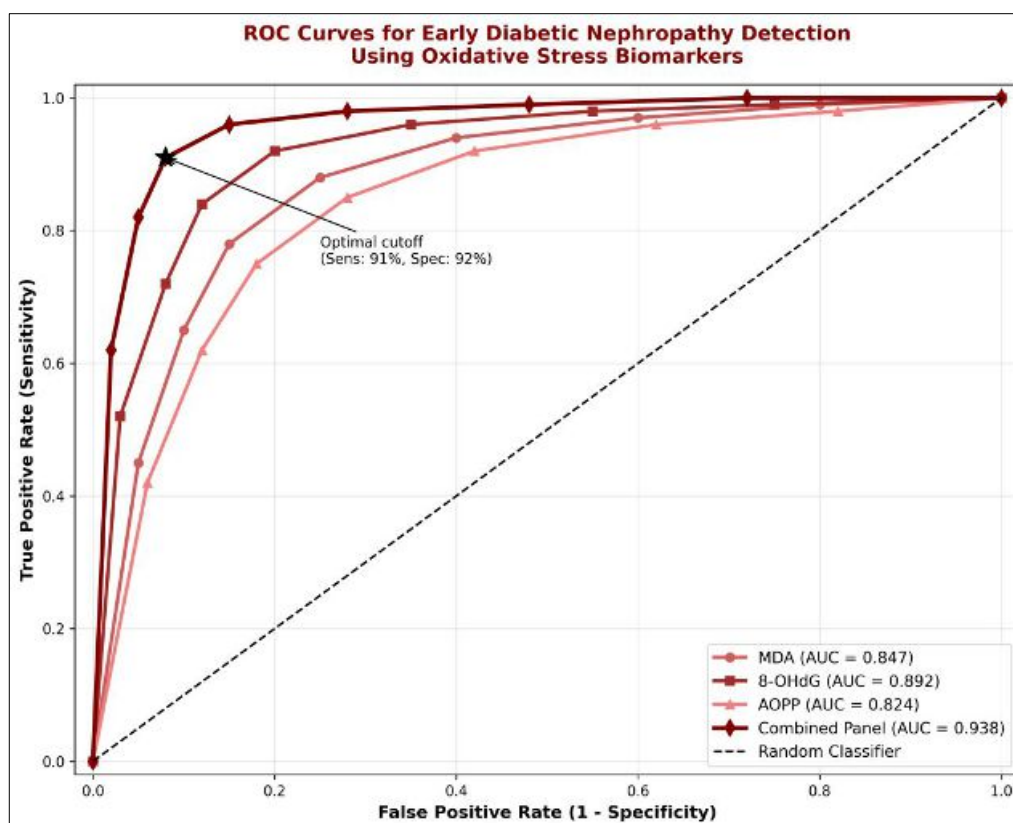


Fig 4: ROC curves for early diabetic nephropathy detection using oxidative stress biomarkers showing superior performance of combined panel (AUC=0.938)

Discussion

This research provides comprehensive characterization of oxidative stress biomarkers across diabetic nephropathy stages in an Argentine population, demonstrating clear associations between oxidative damage accumulation, antioxidant depletion, and renal function decline [19]. The progressive elevation of lipid peroxidation (MDA), protein oxidation (AOPP, carbonyls), and DNA damage (8-OHdG) markers with advancing nephropathy stages confirms oxidative stress as a central pathophysiological mechanism in diabetic kidney disease progression. The magnitude of biomarker changes observed, with 3.4-4.4-fold elevations in advanced compared to healthy subjects, underscores the severity of oxidative imbalance accompanying progressive renal injury.

The parallel decline in antioxidant enzyme activities suggests that oxidative stress in diabetic nephropathy results from both increased ROS generation and compromised antioxidant defenses. SOD activity reductions to approximately one-third of normal values in advanced disease indicate substantial impairment of primary superoxide scavenging capacity. This enzymatic dysfunction may reflect oxidative inactivation of the enzymes themselves, depletion of essential cofactors, or transcriptional downregulation under chronic hyperglycemic conditions [20]. The strong correlations between oxidative markers and eGFR support the utility of these biomarkers for monitoring disease progression and potentially guiding therapeutic interventions targeting oxidative stress pathways.

Conclusion

This research establishes comprehensive reference data for oxidative stress biomarkers across diabetic nephropathy

stages in an Argentine population, confirming progressive oxidative damage accumulation and antioxidant depletion accompanying renal function decline. The excellent diagnostic performance of combined biomarker panels (AUC=0.938) supports clinical utility for early nephropathy detection, potentially enabling earlier intervention and improved outcomes. Future longitudinal research should evaluate these markers as predictors of disease progression and therapeutic response, while intervention trials targeting oxidative stress pathways may benefit from incorporating these biomarkers as efficacy endpoints [21]. Integration of oxidative stress assessment into diabetic nephropathy management algorithms holds promise for personalized risk stratification and treatment optimization in South American diabetic populations.

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