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Oxidative Stress, Antioxidant Depletion, and DNA Damage in Post-COVID-19 Patients: Evidence of a Disrupted Redox Network and Loss of Age-Dependent Antioxidant Compensation

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Abstract

Background

The relationship between oxidative stress and viral infections is well established, yet data on the redox consequences of COVID-19 beyond the acute phase remain sparse. We conducted a comprehensive assessment of oxidative stress biomarkers, antioxidant defences, and DNA damage in post-COVID patients, and examined whether COVID-19 infection disrupts the normal architecture of the antioxidant network.

Methods

In this single-centre, cross-sectional, case-control study, 40 symptomatic post-COVID patients and 40 age- and sex-matched healthy controls were recruited from the Department of Respiratory Medicine, King George's Medical University, Lucknow, India. Blood levels of lipid peroxidation (LPO), total antioxidant activity (TAA), superoxide dismutase (SOD), and glutathione reductase (GR) were measured. DNA damage was quantified using the alkaline comet assay. Data were analysed using Mann-Whitney U tests and Spearman rank correlations.

Results

Post-COVID patients showed profound antioxidant depletion: TAA was reduced by 60.8% (median 51.7 vs 224.3 mM; $p < 0.001$; Cohen's $d = 1.57$), SOD by 34.0% ($p < 0.001$; $d = 0.58$), and GR by 35.0% ($p < 0.001$; $d = 0.65$). LPO was elevated but did not reach significance after correction for non-normality ($p = 0.254$), though it correlated significantly with radiographic severity ($\rho = 0.403$; $p = 0.010$) and was markedly elevated in patients with neurological involvement (2486.6 nmole/ml; $p = 0.008$). DNA damage was significantly increased across all comet parameters (% Tail DNA: +24.0%; $p < 0.001$; $d = 0.82$). A novel finding was that the physiological age-dependent increase in TAA observed in controls ($\rho = 0.425$; $p = 0.006$) was abolished in patients ($\rho = -0.061$; $p = 0.707$). Inter-marker correlation analysis revealed a rewiring of the antioxidant network, with breakdown of the normal LPO–TAA feedback relationship and emergence of SOD–TAA co-depletion.

Conclusions

Post-COVID patients exhibit severe antioxidant depletion, significant DNA damage, and disruption of the normal redox network architecture. These findings provide a biochemical rationale for antioxidant-targeted therapeutic strategies in post-COVID management.

1. Introduction

The COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has affected hundreds of millions of people worldwide since its emergence in Wuhan, China, in late 2019 [1]. While the acute phase of the disease has been extensively studied, a substantial proportion of survivors continue to experience persistent symptoms beyond 12 weeks, a condition termed post-COVID syndrome [2]. These sequelae encompass a broad clinical spectrum, including fatigue, dyspnoea, cognitive impairment, and multi-organ dysfunction, the pathophysiology of which remains incompletely understood.

One of the principal mechanisms by which SARS-CoV-2 causes organ damage is through mitochondrial dysfunction, leading to overproduction of reactive oxygen species (ROS) [3]. Under physiological conditions, ROS are counterbalanced by a tightly regulated antioxidant defence system comprising enzymatic components—superoxide dismutase (SOD), catalase, and glutathione reductase (GR)—and non-enzymatic molecules including vitamins C and E, albumin, and uric acid, collectively reflected by total antioxidant activity (TAA) [4]. When this equilibrium is disrupted, oxidative stress ensues, leading to lipid peroxidation of cell membranes, protein oxidation, and ultimately DNA damage [5].

Although a clear correlation between oxidative stress markers and disease severity has been demonstrated for many viral infections, clinical data for SARS-CoV-2 remain limited [6]. Moreover, existing studies have predominantly focused on the acute phase, examining individual markers in isolation. No study to date has comprehensively assessed the interrelationship between multiple oxidative stress markers, antioxidant defences, and DNA damage in a single cohort of post-COVID patients, nor has the structural integrity of the antioxidant network itself been examined.

We hypothesised that post-COVID patients would exhibit not only elevated oxidative damage and depleted antioxidant defences, but also a disruption of the normal correlational architecture of the redox system. To test this, we measured four oxidative stress biomarkers alongside DNA damage via the alkaline comet assay in 40 post-COVID patients and 40 healthy controls (Fig. 1). We further examined whether COVID-19 infection alters the age-dependent regulation of antioxidant capacity and the inter-marker correlational structure of the redox network.

2. Materials and Methods

2.1 Study Design and Participants

This cross-sectional, comparative, case-control study was conducted at the Department of Respiratory Medicine, King George's Medical University (KGMU), Lucknow, India, in collaboration with the Food Toxicology Laboratory, CSIR–Indian Institute of Toxicology Research (CSIR-IITR), Lucknow. The study was approved by the Institutional Ethics Committee (protocol code 107th ECM II B—Thesis/P8, approved 14 July 2021) and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants.

Forty symptomatic post-COVID patients were enrolled who met the following criteria: age ≥ 18 years; confirmed past history of COVID-19 infection (RT-PCR positive); at least two subsequent negative RT-PCR reports; and persistence of symptoms beyond 4 weeks from testing positive that could not be explained by an alternative diagnosis. Forty age- and sex-matched healthy controls with no history of COVID-19 infection or acute febrile illness in the preceding 3 months and no known comorbid conditions were recruited from the same demographic profile.

2.2 Clinical Assessment

All patients underwent clinical evaluation including assessment of residual symptoms, resting oxygen saturation, and FiO2 requirement at the time of sample collection. Chest radiographs were scored on a scale of 0–18 according to the method described by Monaco et al. [7]. Disease severity during the acute phase was classified as mild, moderate, or severe based on WHO criteria. The presence of comorbidities and involvement of organ systems beyond the respiratory tract (CNS, CVS, GIT, renal, musculoskeletal) were recorded.

2.3 Sample Collection and Biochemical Assays

Ten millilitres of venous blood was collected in EDTA vials and transported to the Food Toxicology Laboratory at CSIR-IITR within 2 hours of collection. **Lipid peroxidation (LPO)** was measured using the malondialdehyde (MDA) assay (Sigma-Aldrich kit MAK085) [8]. **Total antioxidant activity (TAA)** was assessed using the ABTS method. **Superoxide dismutase (SOD)** activity was determined by the method of Sun et al. (1998). **Glutathione reductase (GR)** was measured according to Dringen et al. (1996).

2.4 Comet Assay

DNA damage was assessed using the alkaline comet assay (single-cell gel electrophoresis) according to Singh et al. (1988) [9]. Slides were scored using the Komet 5 image analysis system (Kinetic Imaging, Liverpool, UK) attached to a fluorescence microscope (Leica, Germany) with a 40× objective. Fifty randomly selected cells were scored per sample. Three parameters were recorded: percentage of tail DNA, olive tail moment (OTM, arbitrary units), and tail length (μm).

2.5 Statistical Analysis

All data were tested for normality using the Shapiro-Wilk test. As all four biomarkers in the patient group showed significant departure from normality (all $p < 0.001$), non-parametric methods were used for primary analyses. Case-control comparisons were performed using the Mann-Whitney U test with rank-biserial correlation as the effect size measure. Cohen's d was calculated for standardised effect size interpretation. Spearman rank correlation coefficients were used for all association analyses. Inter-marker correlations were computed separately for patients and controls to examine network-level changes. A p-value < 0.05 was considered statistically significant.

3. Results

3.1 Demographic and Clinical Profile

The patient group comprised 32 males (80%) and 8 females (20%) with a mean age of 48.4 years (range 28–70). The control group comprised 27 males (67.5%) and 13 females (32.5%) with a mean age of 37.6 years (range 26–60). Among patients, 33 (82.5%) had experienced severe COVID, 4 (10%) moderate, and 3 (7.5%) mild disease. Twenty-three patients (57.5%) had comorbidities, with diabetes mellitus being the most common. The most prevalent residual symptoms were fatigue (85%), breathlessness (82.5%), ageusia (75%), and cough (62.5%). Twenty-two patients (55%) had extra-pulmonary system involvement, most commonly cardiovascular (n = 7) and renal (n = 7) followed by CNS (n = 4).

3.2 Oxidative Stress Biomarkers: Case-Control Comparison

Descriptive statistics and case-control comparisons for all four biomarkers are presented in Table 1 and Fig. 2. Given the significant departure from normality in all patient-group markers (Shapiro-Wilk $p < 0.001$ for all), results are presented as both mean ± SD and median (IQR), with Mann-Whitney U as the primary test.

Table 1
Oxidative stress biomarkers in post-COVID patients versus healthy controls.

Marker	Patients (n = 40) Median [IQR]	Controls (n = 40) Median [IQR]	U	p-value	d	Effect
TAA (mM)	51.7 [44.9–76.5]	224.3 [205.2–264.1]	200	**< 0.001**	1.57	Large
SOD (U/ml)	11.5 [6.7–22.8]	26.2 [19.2–37.1]	435	**< 0.001**	0.58	Medium
GR (U/min/mg)	5.0 [2.8–12.1]	14.7 [9.5–23.6]	414	**< 0.001**	0.65	Medium
LPO (nmole/ml)	696.5 [309.8–1369.1]	616.0 [245.4–1159.2]	919	0.254	0.44	Small

Three of four biomarkers showed highly significant differences. TAA demonstrated the largest effect, with a 60.8% reduction in patients relative to controls (Cohen's d = 1.57, large effect). SOD and GR were reduced by 34.0% and 35.0% respectively, both with medium effect sizes. LPO was elevated by 61.6% in mean values; however, extreme variability in the patient group (coefficient of variation = 120.6%) meant that the median difference was modest and the Mann-Whitney U test did not reach significance ($p = 0.254$).

3.3 DNA Damage

All three comet assay parameters were significantly elevated in post-COVID patients (Table 2, Fig. 3). Percentage tail DNA showed the largest effect (d = 0.82), representing a 24.0% increase in DNA fragmentation.

Table 2
DNA damage parameters by alkaline comet assay.

Parameter	Patients (mean ± SE)	Controls (mean ± SE)	t	p-value	d
% Tail DNA	12.50 ± 0.49	10.08 ± 0.44	3.675	**< 0.001**	0.82
OTM (arb. units)	1.26 ± 0.06	1.03 ± 0.05	2.945	**0.004**	0.66
Tail length (μm)	14.90 ± 0.36	13.18 ± 0.50	2.792	**0.007**	0.62

3.4 LPO and Clinical Correlates

Although LPO did not differ significantly between groups overall, subgroup analyses revealed clinically meaningful associations (Fig. 4). LPO increased in a dose-dependent fashion with COVID severity (mild: 579.8; moderate: 707.8; severe: 1262.7 nmole/ml) and correlated significantly with chest radiograph severity score (Spearman ρ = 0.403; p = 0.010), demonstrating a 3.4-fold increase from the lowest to highest score category. LPO was significantly higher in patients with comorbidities (p = 0.032) and showed a striking gradient across organ systems, with patients exhibiting neurological sequelae showing the highest levels (2486.6 nmole/ml; p = 0.008)—over twice the cohort mean (Table 3).

Table 3
Lipid peroxidation stratified by clinical and radiological variables.

Clinical Variable	LPO (nmole/ml)	p-value
Severity: Mild	579.8	—
Severity: Moderate	707.8	—
Severity: Severe	1262.7	**0.032**
X-ray score 0–6	518.3	—
X-ray score 6–12	1041.6	—
X-ray score 12–18	1744.2	**0.010**
With comorbidities	1156.0	—
Without comorbidities	715.6	**0.032**
CNS involvement	2486.6	**0.008**
CVS involvement	1230.1	**0.002**
Renal involvement	1325.5	**0.010**
Musculoskeletal	1018.3	0.299

3.5 Disruption of Age-Dependent Antioxidant Compensation

In healthy controls, TAA increased significantly with age (Spearman ρ = 0.425; p = 0.006), consistent with a physiological compensatory upregulation of antioxidant capacity (Fig. 5A). This relationship was completely abolished in post-COVID patients (ρ = −0.061; p = 0.707; Fig. 5B). No other marker showed a significant age-dependent relationship in either group (Table 4).

Table 4
Spearman correlations between age and oxidative stress markers.

Marker	Patient ρ	Patient p	Control ρ	Control p
TAA	-0.061	0.707	**0.425**	**0.006**
LPO	-0.012	0.941	-0.025	0.878
SOD	-0.064	0.695	-0.064	0.693
GR	0.030	0.852	0.025	0.878

3.6 Rewiring of the Antioxidant Network

Inter-marker Spearman correlations computed separately for patients and controls revealed two notable shifts (Table 5, Fig. 6). In controls, LPO and TAA showed a significant positive correlation (ρ = 0.344; p = 0.030), consistent with a homeostatic feedback loop. This relationship was absent in patients (ρ = −0.114; p = 0.484). Conversely, a new correlation between TAA and SOD emerged in patients (ρ = 0.444; p = 0.004) that was absent in controls (ρ = 0.157; p = 0.334), suggesting co-depletion of enzymatic and non-enzymatic antioxidant pools.

Table 5
Inter-marker Spearman correlations in patients versus controls.

Marker Pair	Patient ρ	p	Control ρ	p
LPO vs TAA	-0.114	0.484	**0.344**	**0.030**
LPO vs SOD	-0.090	0.579	0.094	0.565
LPO vs GR	-0.013	0.937	0.047	0.776
TAA vs SOD	**0.444**	**0.004**	0.157	0.334
TAA vs GR	0.143	0.378	0.126	0.440
SOD vs GR	0.129	0.427	0.030	0.854

3.7 Sex-Stratified Vulnerability

Sex-stratified analysis revealed dimorphic patterns of oxidative damage (Fig. 7). Female patients showed a 108.7% increase in LPO compared to female controls, versus 47.4% in males. Conversely, males showed more severe TAA depletion (− 66.9%) compared to females (− 36.6%). SOD reduction was comparable between sexes.

4. Discussion

This study provides the most comprehensive assessment to date of the oxidative stress–antioxidant balance in post-COVID patients, combining four biomarkers with DNA damage analysis and, for the first time, examining the structural integrity of the redox network itself. Our principal findings are fourfold: (i) profound and consistent antioxidant depletion across multiple pathways; (ii) significant DNA damage indicating genotoxic consequences; (iii) disruption of the physiological age-dependent antioxidant compensation; and (iv) rewiring of the inter-marker correlational architecture of the redox system.

The most robust finding was the severe depletion of total antioxidant activity, which showed a large effect size ($d = 1.57$) and the highest statistical significance. This is consistent with the findings of Martín-Fernández et al. (2021), who reported lower total antioxidant capacity in acute COVID-19 patients [10], and extends these observations to the post-acute phase. The concurrent reduction in both enzymatic (SOD, GR) and non-enzymatic (TAA) antioxidant defences suggests exhaustion of the entire antioxidant system rather than dysfunction of a single pathway.

An important methodological consideration is the non-significance of lipid peroxidation in the overall case-control comparison when appropriate non-parametric testing is applied. The extreme variability in patient LPO values (CV = 120.6%) renders the mean a poor summary statistic. However, the clinical relevance of LPO is preserved in subgroup analyses: it correlated significantly with radiographic severity ($\rho = 0.403$; $p = 0.010$) and was strikingly elevated in patients with neurological sequelae (2486.6 nmole/ml; $p = 0.008$). The latter finding is particularly noteworthy: patients with CNS involvement had LPO levels 2.15 times the cohort average, suggesting that the brain may be especially vulnerable to post-COVID lipid peroxidative damage. This aligns with evidence that the CNS is rich in polyunsaturated fatty acids and has limited antioxidant reserve [11].

Our demonstration of significant DNA damage via the comet assay provides direct evidence of genotoxic consequences of post-COVID oxidative stress. The 24% increase in tail DNA percentage ($d = 0.82$, large effect) indicates substantial DNA strand breakage persisting beyond the acute infection. While comet assays have documented DNA damage in other chronic diseases [12], ours is among the first to demonstrate this specifically in post-COVID patients.

Perhaps the most novel findings relate to the network-level disruption of the antioxidant system. The abolition of the age–TAA relationship is striking: in healthy controls, antioxidant capacity increases with age, likely reflecting a compensatory response to age-related oxidative burden. This mechanism is

entirely disrupted by COVID-19, suggesting that the virus overwhelms not merely the quantity but the regulatory architecture of the antioxidant system. The rewiring of inter-marker correlations further supports this: the normal LPO–TAA feedback loop breaks down, replaced by SOD–TAA co-depletion—a transition from compensatory to uncompensated oxidative stress.

The sex-dimorphic pattern of oxidative damage deserves attention. The disproportionate LPO elevation in females (+ 108.7% vs + 47.4% in males) contrasts with the more severe TAA depletion in males (− 66.9% vs − 36.6%), suggesting sex-specific patterns of oxidative vulnerability that may inform personalised therapeutic approaches.

Our study has several limitations. The sample size of 40 per group limits statistical power for subgroup analyses, particularly the severity subgroups where mild (n = 3) and moderate (n = 4) groups are small. The cross-sectional design precludes assessment of temporal dynamics. The extreme variability in patient LPO warrants caution in interpreting mean values.

5. Conclusions

Post-COVID patients exhibit severe, multi-pathway antioxidant depletion, significant DNA damage, and fundamental disruption of the redox network architecture. The loss of age-dependent antioxidant compensation and the shift from compensatory to co-depleting inter-marker dynamics represent novel findings that advance our understanding of the pathophysiology of post-COVID syndrome. These data provide a biochemical rationale for antioxidant-targeted interventions and highlight the need for long-term monitoring of genotoxic consequences in COVID-19 survivors.

Declarations

Ethics Approval and Consent to Participate: This study was performed after approval from the Institutional Ethics Committee, King George's Medical University, Lucknow, Uttar Pradesh, India (Ref. code: 107th ECM IIB-Thesis/P8; No. 840/Ethics/2021; dated 14/07/2021). All procedures performed in this study involving human participants were conducted in accordance with the ethical standards of the institutional and/or national research committee and with the principles outlined in the Declaration of Helsinki.

Consent to Participate: Informed consent was obtained from all individual participants included in the study. Participants were fully informed about the purpose of the research, the procedures involved, potential risks and benefits, and their right to withdraw at any time without penalty. Confidentiality and anonymity of participants was strictly maintained throughout the study. No personally identifiable information has been disclosed in this research.

Consent for Publication: Not applicable.

Clinical Trial Number: Not applicable.

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Conflict of Interest: The authors declare that they have no competing financial or non-financial interests.

Data Availability Statement: The data supporting the findings of this study are available upon request from the corresponding author. The data are not publicly available due to privacy/ethical restrictions.

Acknowledgements: Nil.

Author Contributions: N.D. wrote the original draft and conducted the investigation. S.K.T. conceptualized the study and supervised the research. J.B. reviewed and edited the manuscript. A.V. provided methodology and resources. K.M.A. supervised the laboratory investigation at CSIR-IITR. C.K. and I.J. performed the laboratory assays and data acquisition. S.K.V., S.K., R.A.S.K., R.G., A.S., D.B., and A.K. contributed to data collection, clinical evaluation, and critical review of the manuscript. All authors reviewed the manuscript and approved the final version. The authors declare that all data were generated in-house, that no paper mill was used and that no AI tool has been used for the generation of text or figures.

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Figures

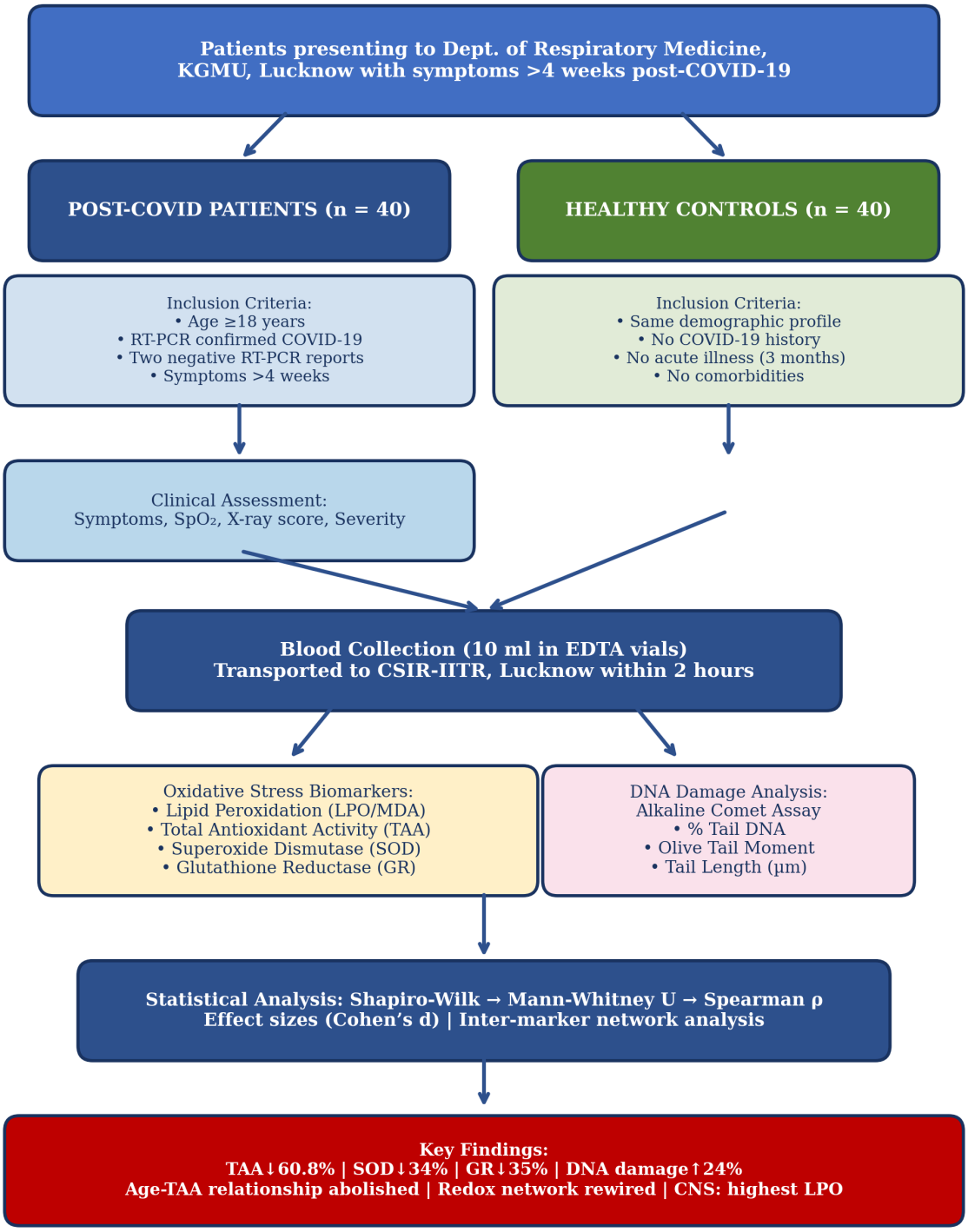


Figure 1

Study flow diagram showing enrollment, clinical assessment, laboratory assays, and statistical analysis pipeline.

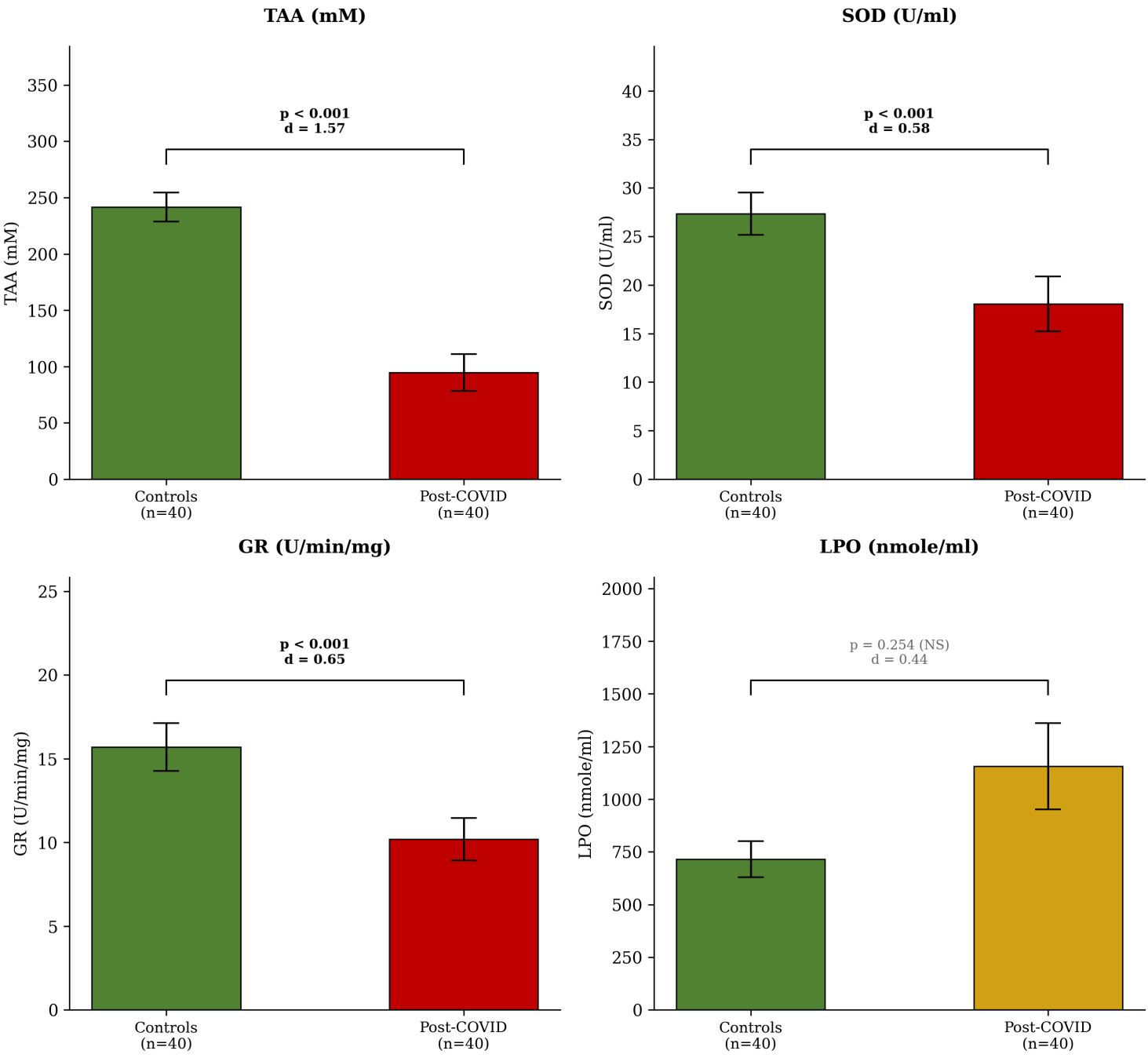


Figure 2

Oxidative stress biomarkers in post-COVID patients (red) versus healthy controls (green). Error bars represent SEM. Significance brackets show Mann-Whitney p-values and Cohen's d effect sizes.

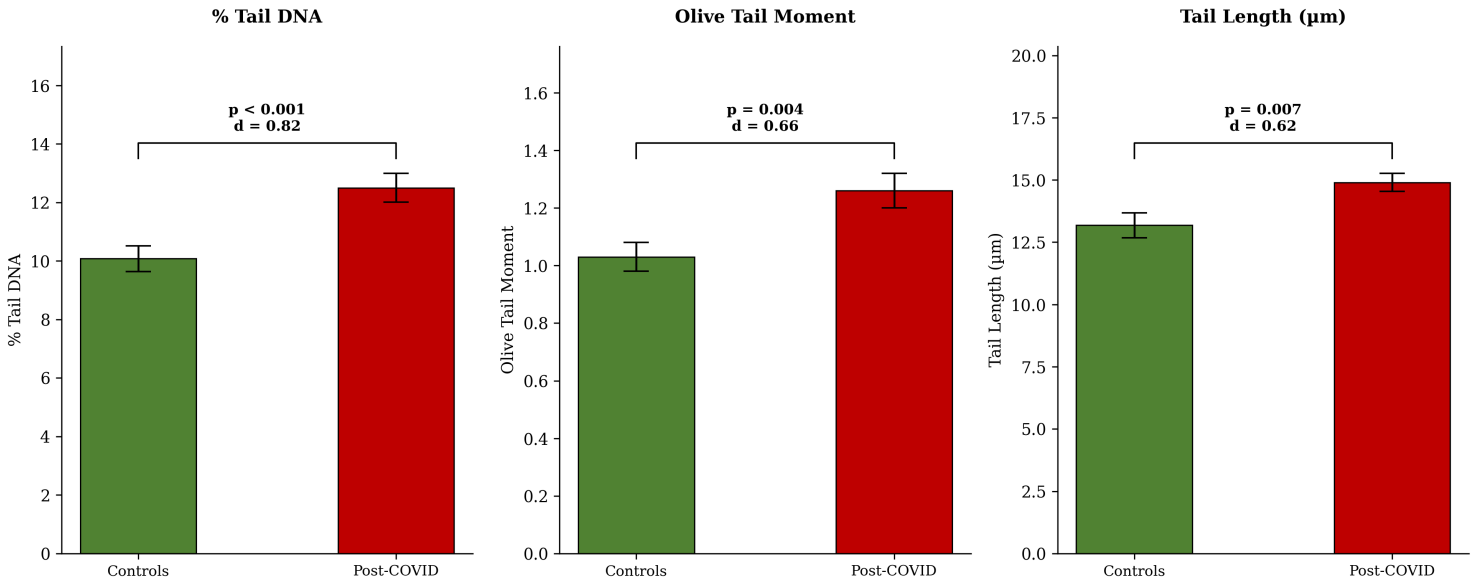


Figure 3

DNA damage parameters (comet assay) in post-COVID patients versus controls. All three parameters were significantly elevated.

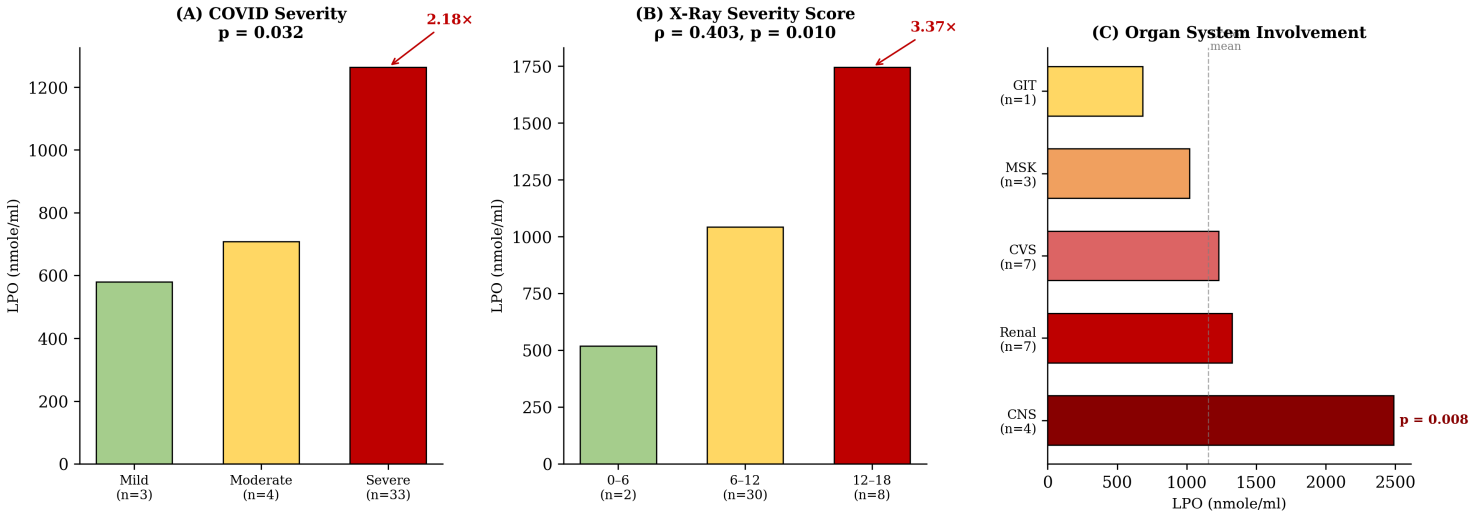


Figure 4

Lipid peroxidation stratified by (A) COVID severity, (B) chest X-ray severity score, and (C) organ system involvement. Dashed line in (C) indicates cohort mean.

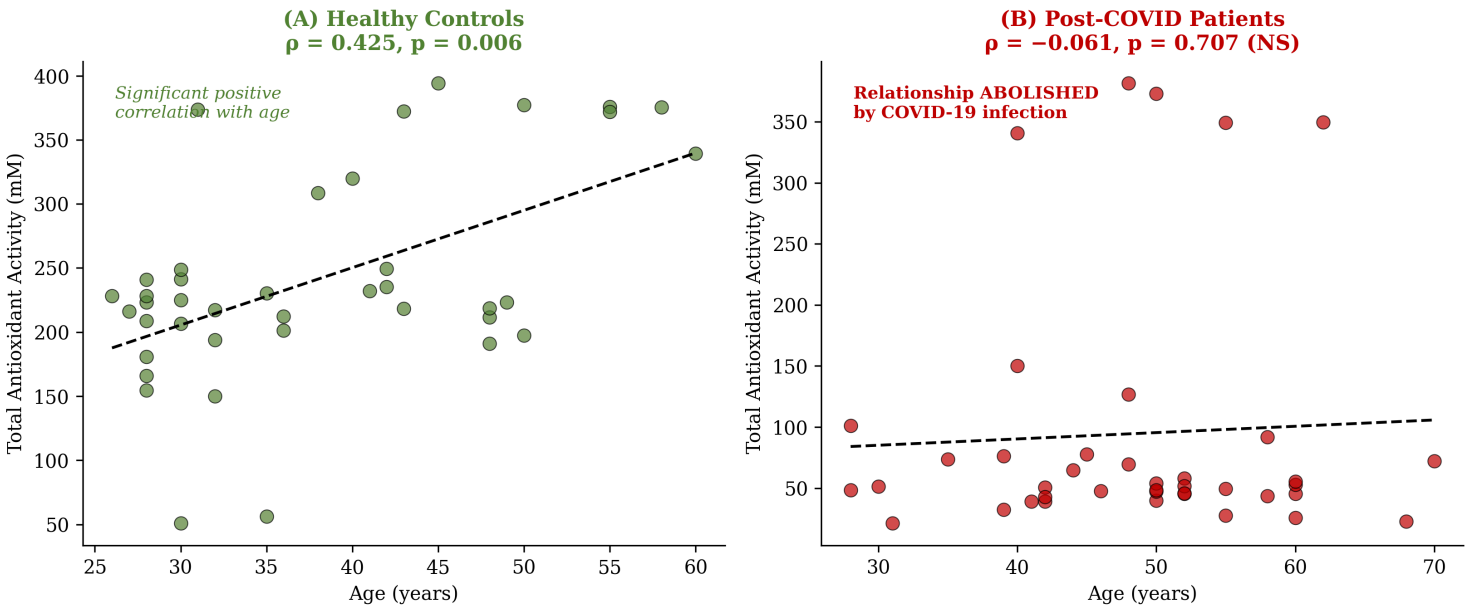


Figure 5

Disruption of age-dependent antioxidant compensation. (A) Healthy controls show a significant positive correlation between age and TAA (ρ = 0.425, p = 0.006). (B) This relationship is abolished in post-COVID patients (ρ = −0.061, p = 0.707).

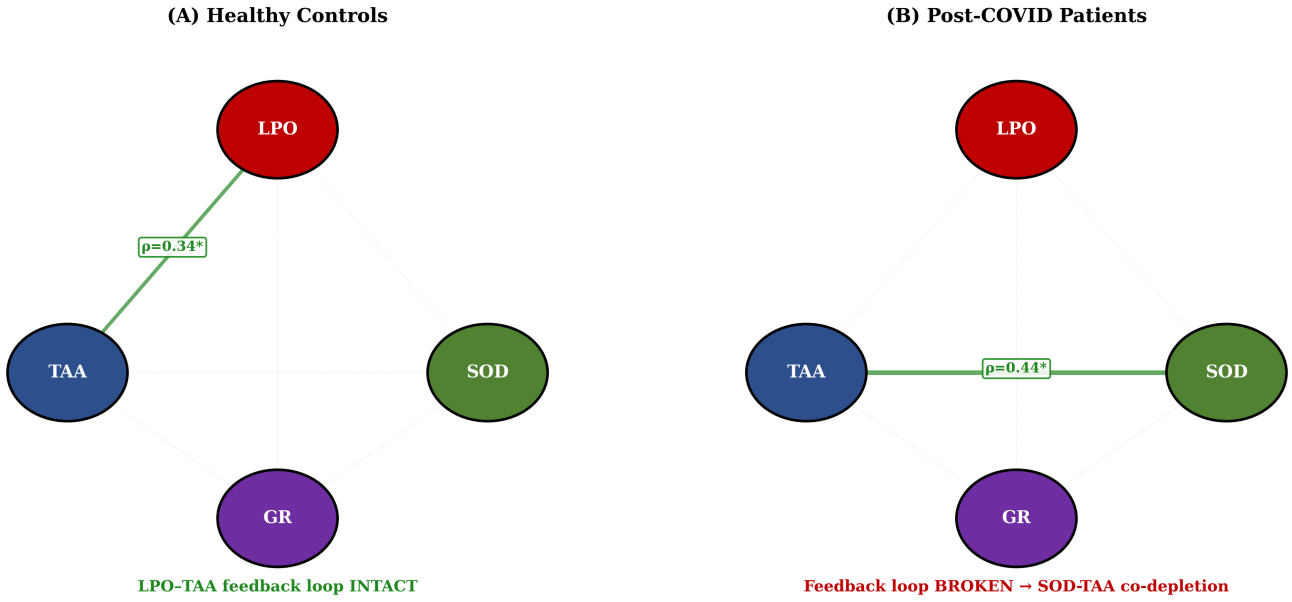


Figure 6

Rewiring of the antioxidant network. (A) In controls, the LPO–TAA feedback loop is intact (green line). (B) In patients, this loop is broken and replaced by SOD–TAA co-depletion (red line). Line thickness proportional to correlation strength.

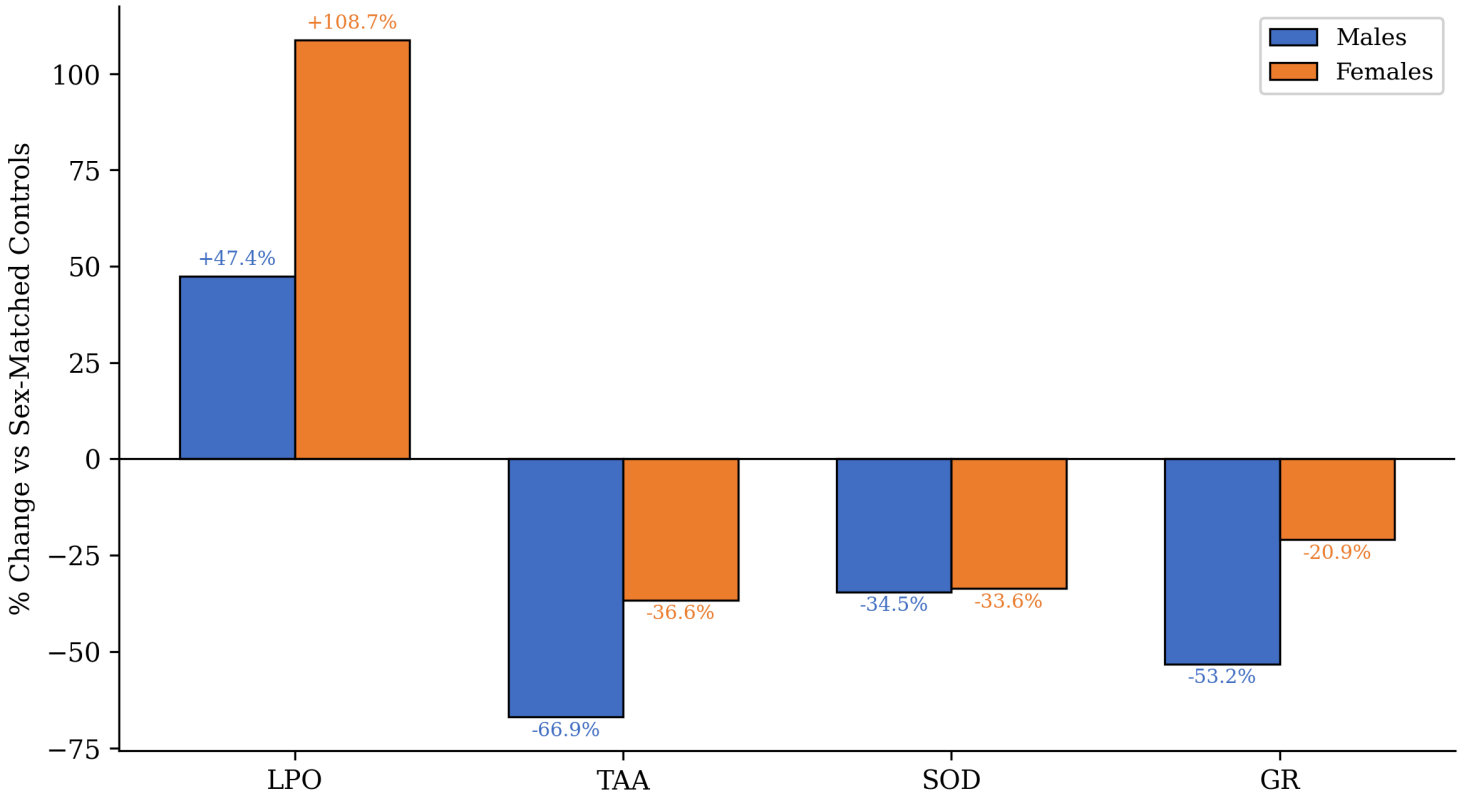


Figure 7

Sex-stratified percentage change in biomarkers (post-COVID vs sex-matched controls). Females show disproportionate LPO elevation; males show more severe TAA depletion.

Supplementary Files

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- supplementarymaterial.docx