



TREATMENT EFFECTS ON THE ROS PRODUCTION, LIPID PEROXIDATION AND OXIDATIVE DAMAGES IN IBD PEDIATRIC PATIENTS

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ABSTRACT:

Background: Inflammatory bowel disease (IBD) is a chronic immune-mediated disorder encompassing Crohn’s disease (CD) and ulcerative colitis (UC). Increasing evidence suggests that impaired intestinal immune regulation in IBD is closely linked to an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, resulting in oxidative stress. Excessive ROS generation and lipid peroxidation contribute to mucosal injury, activation of inflammatory signaling pathways, and disease progression.

Objective: The aim of this study was to evaluate the role of oxidative stress markers—ROS production and malondialdehyde (MDA)—measured in peripheral blood, as well as antioxidant status, in relation to disease course and treatment in patients with IBD.

Materials and Methods: A cross-sectional study was conducted including 21 children and young adults with IBD (aged 14–25 years; mean age ~17.1 years) and 22 age-matched healthy controls (mean age ~17.6 years). Among the IBD cohort, 9 patients (42%) were male and 12 (58%) were female; 7 patients were diagnosed with CD and 14 with UC. All IBD patients underwent histological assessment.

Results: Increased ROS production was positively correlated with elevated MDA levels, indicating enhanced lipid peroxidation and oxidative stress in patients with IBD.

Conclusions: The therapeutic efficacy of anti-inflammatory treatments, including azathioprine, corticosteroids, mesalazine, and biological agents, may be partly attributed to their capacity to reduce ROS production and lipid peroxidation in intestinal mucosal cells, thereby promoting mucosal healing in patients with IBD.

Keywords: IBD, ROS production, lipid peroxidation, anti-inflammatory drug efficacy,

INTRODUCTION

Inflammatory bowel disease (IBD) has increased markedly over the past decade, particularly among children and adolescents. IBD is a chronic, immune-mediated disorder that includes Crohn’s disease (CD) and ulcerative colitis (UC), both affecting the gastrointestinal tract. CD is characterized by discontinuous (“skip”) transmural inflammation, most commonly involving the ileum and colon, and is frequently associated with granulomas, strictures, fistulas, abscesses, and intestinal obstruction. In contrast, UC is limited to the superficial mucosal layers of the colon and rectum—regions with dense microbial colonization [1, 2].

A growing body of evidence implicates redox imbalance and excessive production of reactive oxygen species (ROS) in colonic tissues as key contributors to IBD pathogenesis. Oxidative stress induces structural and functional damage to lipids, proteins, and DNA, disrupts intracellular signaling, promotes mucosal infiltration by activated phagocytes, and amplifies inflammatory responses, thereby facilitating disease progression and exacerbation [3, 4]. ROS, which arise as by-products of aerobic metabolism, include superoxide anion ($\bullet\text{O}_2^-$), nitric oxide ($\bullet\text{NO}$), hydroxyl radical ($\bullet\text{OH}$), hydroperoxyl radical ($\bullet\text{O}_2\text{H}$), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1\text{O}_2$) [5, 6].

In IBD, excessive ROS accumulation particularly affects cellular membrane lipids rich in polyunsaturated fatty acids (PUFAs), leading to uncontrolled lipid peroxidation and the formation of lipid peroxyl radicals (LOOH/LOO \bullet) [7]. These processes disrupt membrane architecture and lipoprotein interactions in intestinal epithelial cells, resulting in the generation of reactive aldehydes such as malondialdehyde (MDA), which can further potentiate inflammatory signaling pathways, including Toll-like receptor 4 activation [7, 8].

Secondary products of lipid peroxidation may also form covalent adducts with amino acid residues, including lysine, histidine, and cysteine, thereby impairing protein structure and function [8]. Collectively, these oxidative modifications compromise epithelial membrane integrity and weaken the intestinal barrier, increasing permeability and facilitating the translocation of luminal antigens and microbial products into underlying tissues. This process triggers

inappropriate immune activation and sustains chronic intestinal inflammation characteristic of IBD [3, 6, 8, 9].

Activated phagocytic immune cells infiltrating the intestinal mucosa further exacerbate oxidative stress through enhanced ROS generation, leading to increased levels of superoxide anion ($\bullet\text{O}_2^-$) and hydroxyl radicals ($\bullet\text{OH}$). These radicals promote oxidative chain reactions via the Haber–Weiss and Fenton pathways [10]. In parallel, hydroxyl and hydroperoxyl radicals contribute to mucin depolymerization, mitochondrial dysfunction, and oxidative damage to mitochondrial RNA and DNA, as well as lipid peroxidation of gastrointestinal lipoproteins [11].

Under physiological conditions, enterocytes of the small intestine maintain relatively low ROS levels, whereas colonic enterocytes exhibit significantly higher basal ROS production [12]. The resulting oxidative modifications often form stable biomolecular adducts that serve as measurable biomarkers of ROS generation, lipid peroxidation, and overall oxidative stress status.

Based on these considerations, the aim of the present study was to investigate and compare ROS production and lipid peroxidation levels in pediatric patients with CD and UC, both during disease progression and in response to treatment.

MATERIALS AND METHODS:

Ethical approval

The study was approved by the Ethics Committees of Trakia University Hospital and University Hospital “Prof. Stoyan Kirkovich,” Stara Zagora, Bulgaria, in accordance with the Declaration of Helsinki (approval code: 10-816/12 October 2019). Written informed consent was obtained from all participants or their legal guardians prior to enrollment.

Study population

This study included patients admitted to the participating clinics between January 2023 and February 2025 with symptoms suggestive of inflammatory bowel disease, including diarrhea, bloody stools, and cramping abdominal pain. The diagnosis of Crohn’s disease (CD) or ulcerative colitis (UC) was established based on clinical presentation, physical examination, endoscopic findings, and histopathological evaluation (Table 1).

Disease activity was assessed using the Harvey–Bradshaw Index for CD [13] and the Rachmilewitz Index for UC [14]. Inflammatory status was further evaluated using standard laboratory markers, including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), platelet and leukocyte counts, fecal calprotectin, hemoglobin, and serum albumin levels [15].

A total of 21 children and young adults with IBD (aged 14–25 years; mean age ~17.1 years) were enrolled. The cohort included 9 males (42%) and 12 females (58%). Seven patients were diagnosed with active CD and fourteen with active UC. All IBD patients underwent histological confirmation of diagnosis.

The control group consisted of 22 age-matched healthy individuals (mean age ~17.6 years) who were non-smokers, not receiving medication, without comorbidities,

and with no clinical signs, symptoms, or history of IBD or other inflammatory diseases.

Inclusion and exclusion criteria

Inclusion criteria for IBD patients were: positive colonoscopic findings with histological confirmation and elevated fecal calprotectin levels. Exclusion criteria were: evidence of intestinal infection and presence of other acute infectious conditions.

Treatment regimens

All patients included in the study were receiving one or more of the following treatments for IBD: corticosteroids (40 mg/day), azathioprine (100 mg/day), mesalazine (3 g/day), and, in selected cases, biological therapy (infliximab or adalimumab).

Sample collection and laboratory analyses

After a 12-hour overnight fast, venous blood samples (1.5 mL) were collected from both IBD patients and controls at 7:30 a.m. Serum was separated by centrifugation (2000×g, 10 min, 4°C) and analyzed immediately. Complete blood count, ESR, CRP, reactive oxygen species (ROS) production, and lipid peroxidation assessed as malondialdehyde (MDA) levels were measured.

Antioxidant enzyme activity was evaluated by measuring glutathione peroxidase-1 (GPx-1; ELISA kit No. ab41464), while lipid peroxidation was assessed via MDA levels (ELISA kit No. ab233471), according to the manufacturers’ instructions.

ROS and oxidative stress measurements

Total ROS production in serum was determined following the method described by Shi et al. [16]. Briefly, 100 μL of serum was mixed with 900 μL of 50 mM N-tert-butyl- α -phenylnitron (PBN) dissolved in dimethyl sulfoxide (DMSO), centrifuged at 4000×g for 10 min at 4 °C, and analyzed by electron paramagnetic resonance (EPR) spectroscopy.

Serum superoxide anion ($\bullet\text{O}_2^-$) levels were measured using the spin trap CMH (1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine), based on the method of Perrone et al. [17]. Briefly, 30 μL of serum was mixed with 30 μL of CMH (1:1) on ice, incubated for 5 min, and subsequently analyzed by EPR.

Protein oxidation was assessed by evaluating albumin thiol (–SH) group modifications using spin conjugation with 3-maleimido-proxyl (5-MSL). Serum samples (100 μL) were mixed with 20 mM 5-MSL dissolved in 900 μL DMSO and centrifuged at 1000 rpm for 15 min at 4°C. Albumin conformational changes were recorded in triplicate using EPR spectroscopy with slide modification, as previously described [18].

EPR spectroscopy

All EPR measurements were performed five times per sample. Spectral acquisition parameters included a center field of 3503–3515 G, microwave power of 6.42–20.00 mW, and modulation amplitude of 5–10 G per sample. Results are expressed in arbitrary units (a.u.).

STATISTICAL METHODS:

Statistical analyses were performed using GraphPad Prism software (version 8.0.1 for Windows; GraphPad Soft-

ware Inc., San Diego, CA, USA). Comparisons between two independent groups were conducted using Student's *t*-test. For analyses involving multiple group comparisons, one-way analysis of variance (ANOVA) was applied. A *p*-value ≤ 0.05 was considered statistically significant.

EPR spectral processing was carried out using WIN-EPR SimFonia software (version 1.2/6130860). Quantitative EPR data were subjected to the same statistical criteria, with statistical significance defined as *p* ≤ 0.05 .

RESULTS:

The diagnosis of inflammatory bowel disease was confirmed in all 21 patients based on a combination of clinical presentation (100%), biochemical markers (93%), endoscopic findings (100%), and histopathological criteria (91%). Key laboratory abnormalities included reduced serum albumin levels and elevated C-reactive protein (CRP), along with markedly increased fecal calprotectin concentrations in all patients. Mean fecal calprotectin levels were approximately 1290 $\mu\text{g/g}$ in patients with Crohn's disease (CD; *n* = 7) and 1820 $\mu\text{g/g}$ in patients with ulcerative colitis (UC; *n* = 14), with a statistically significant difference compared with controls (*p* < 0.001).

All patients underwent colonoscopic evaluation, which revealed characteristic mucosal abnormalities con-

sistent with IBD. Histological examination of biopsy specimens further confirmed typical inflammatory changes. At the time of evaluation, 17 patients (81.0%) were classified as having active disease, while 4 patients (19.0%) were in remission (*p* < 0.005). Among patients with active disease, 6 individuals with CD (85.7%) and 11 with UC (78.6%) were identified (*p* < 0.005). In contrast, remission was observed in 1 patient with CD (14.3%) and 3 patients with UC (21.4%) (*p* < 0.002).

All treatment-naïve patients received therapy in accordance with established clinical guidelines. Patients with CD were primarily treated with immunosuppressive regimens, including azathioprine (100 mg/day; *n* = 7) and corticosteroids (40 mg/day; *n* = 5). Patients diagnosed with UC were treated with mesalazine (3 g/day), either as monotherapy or in combination with immunosuppressive agents. Biological therapy (infliximab or adalimumab) was required in only three patients across both CD and UC groups to achieve adequate inflammatory control.

Comparative analysis revealed no significant differences between CD and UC groups with respect to sex distribution, median age, disease duration, diagnostic delay, or baseline inflammatory activity (Table 1). Extraintestinal manifestations were rare; only one patient with CD (2.09%) presented with associated arthritis.

Table 1. Demographic and clinical characteristics of patients with inflammatory bowel disease (IBD)—Crohn's disease (CD, *n* = 7) and ulcerative colitis (UC, *n* = 14)—and healthy controls (*n* = 22). Comparisons between independent groups were performed using Student's *t*-test.

Demographic and clinical characteristics	CD (n= 7)	UC (n= 14)	Controls (n=22)
Gender: male	4 (57.1%)	5 (35.7%)	9 (42.9%)
female	3 (42.9%)	9 (64.3%)	13 (59.6%)
Age (years) :	15.8± 0.01	20.4± 0.09	17.6 ± 0.07
BMI (kg/m²)	≤+1SD, ~20,9%	≤+1SD, ~19,7%	22.9
Disease duration	~2.6 years	3 years	-
Diagnostic delay (months)	9	3	-
Familial IBD yes/no	No	No	No
Active IBD	6 (85.7%)	11 (78.6%)	-
Remission	1 (14.3%)	3 (21.4%)	-
Fecal calprotectin $\mu\text{g/g}$	~1290	~1820	-
Disease type, n (%)	7 (33.3%)	14 (66.7 %)	-
Disease location (n):			
Ileum	6	0	-
Colon	0	14	-
Ileo-colon	1	0	-
Disease behavior (n):			
Inflammatory	6	14	-
Stricturing	1	0	-
Fistulizing	0	0	-

Extra-intestinal disease (n):			
Anemia	1	1	-
Skin	1	0	-
Arthritis	1	0	-
Pharmacotherapy (n)*			
Mesalazine (3 g/ day)	-	14	-
Corticosteroids (40 mg/day)	5	8	-
Asathioprine (100 mg/day)	7	1	-
Biologics	1	2	-

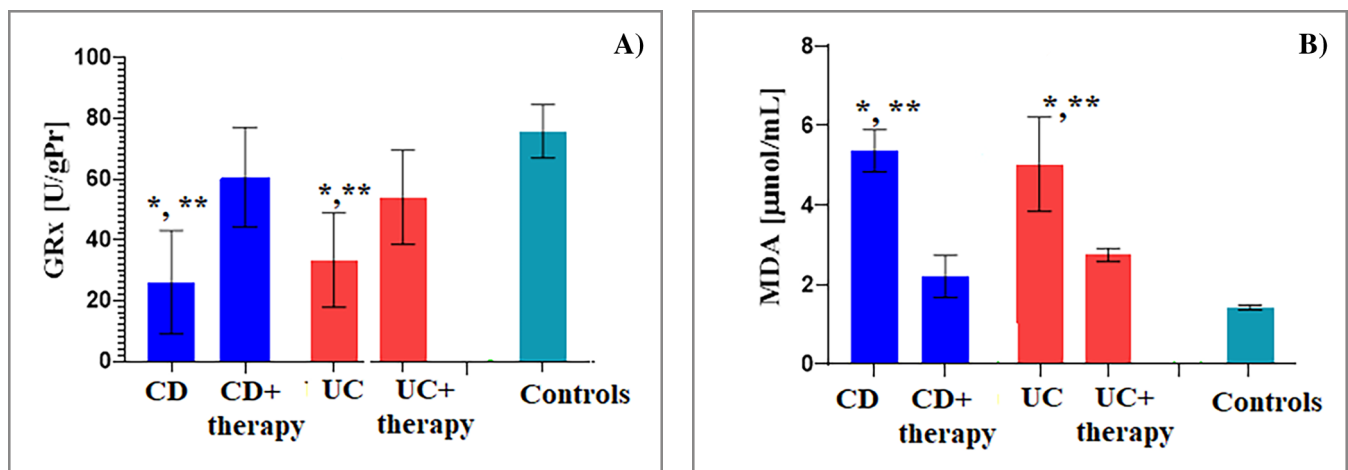
* Some patients received more than one pharmacological agent concurrently. Statistical significance was defined as $p < 0.05$.

According to international WHO age- and sex-specific BMI reference standards, no significant differences in BMI were observed between the study groups. Elevated ambulatory fecal calprotectin levels (mean $\sim 1290 \mu\text{g/g}$) together with positive colonoscopic findings in all 21 patients were used to confirm the diagnosis of IBD and to distinguish between CD and UC.

Serum glutathione peroxidase-1 (GPx-1) levels were significantly lower in both CD and UC patient groups com-

pared with healthy controls ($p < 0.003$). In contrast, no statistically significant differences were observed in plasma malondialdehyde (MDA) levels between IBD patients and controls ($p > 0.05$). Notably, serum GPx-1 levels in CD and UC patients receiving immunosuppressive therapy (azathioprine and/or corticosteroids) were significantly higher than those in untreated CD and UC patients ($p < 0.05$) and exceeded levels observed in the control group ($p < 0.05$) (Figure 1A).

Fig. 1. Effects of therapy on oxidative stress markers in patients with Crohn's disease (CD) and ulcerative colitis (UC): (A) antioxidant glutathione peroxidase-1 (GPx-1) activity and (B) lipid peroxidation assessed by malondialdehyde (MDA) levels. Data are presented as mean \pm standard error (SE). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Student's t -test. $p < 0.05$ vs. control group; $p < 0.001$ vs. corresponding groups before treatment.



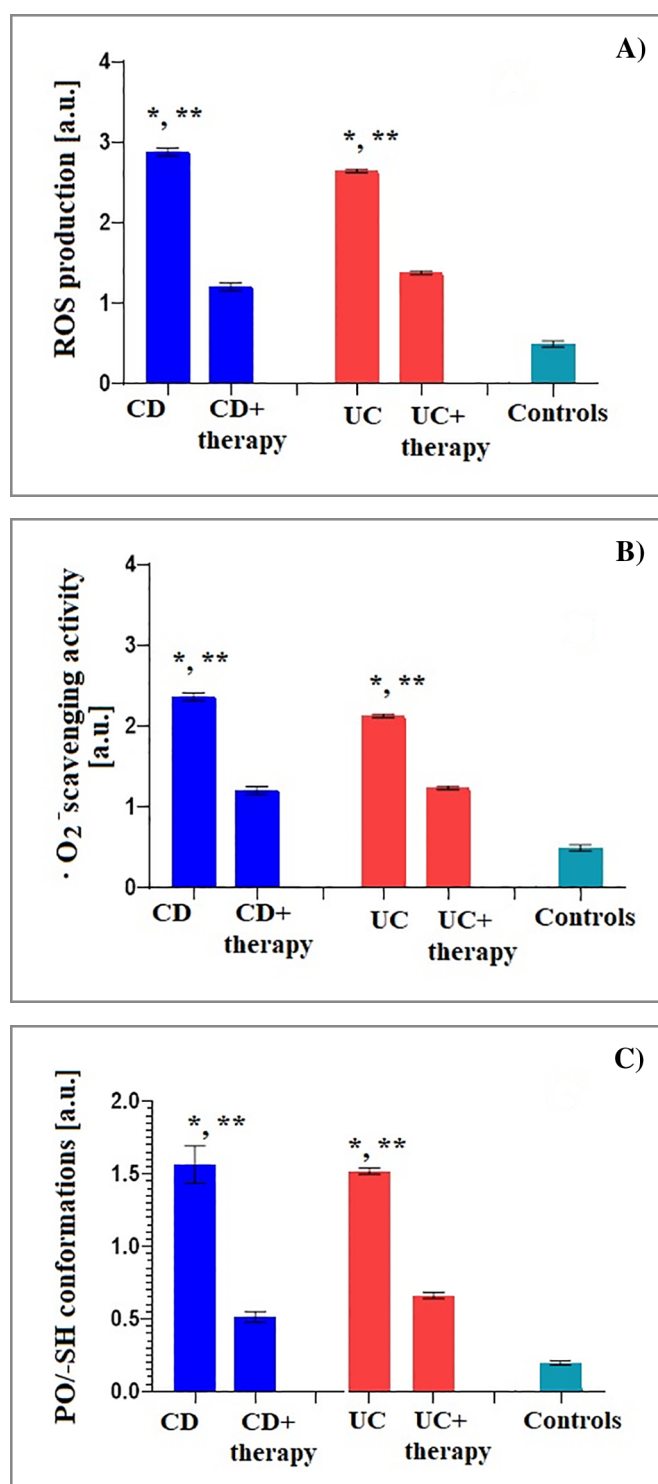
Serum MDA levels did not differ significantly between the UC and CD groups ($p > 0.05$). However, MDA concentrations in both CD and UC patients receiving azathioprine or corticosteroid therapy were approximately two-fold lower compared with untreated CD and UC patients ($p < 0.001$) (Figure 1B).

Serum ROS production was significantly increased in both CD and UC patients, irrespective of disease activity status (active or remission), compared with healthy controls ($p < 0.005$) (Figure 2A). Among treated patients, ROS production remained significantly elevated in CD patients receiving azathioprine and/or corticosteroids,

whereas UC patients receiving the same therapies exhibited an approximately 2.7-fold reduction in ROS levels compared with untreated UC patients ($p < 0.005$).

The mean serum superoxide anion ($\bullet\text{O}_2^-$) concentration was significantly higher in patients with CD ($14.61 \pm 4.94 \mu\text{mol/L}$) compared with healthy controls ($p = 0.048$) and with patients with UC ($p < 0.05$). Elevated $\bullet\text{O}_2^-$ levels were also observed in male patients with UC ($20.12 \pm 5.71 \mu\text{mol/L}$) (Figure 2B). In contrast, no significant differences in $\bullet\text{O}_2^-$ concentrations were detected between CD and UC patients treated with immunosuppressive therapy ($p > 0.05$).

Fig. 2. Effects of therapy on oxidative stress parameters in patients with Crohn's disease (CD) and ulcerative colitis (UC): (A) reactive oxygen species (ROS) production, (B) superoxide anion radical ($\bullet\text{O}_2^-$), and (C) protein oxidation assessed by thiol ($-\text{SH}$) conformational changes. Radical species were measured in triplicate by electron paramagnetic resonance (EPR) spectroscopy using WinEPR and SimFonia software and are expressed in arbitrary units (a.u.). Data are presented as mean \pm standard error (SE). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Student's *t*-test. $p < 0.05$ vs. controls; $p < 0.005$ vs. corresponding groups before treatment.



Protein modification and oxidation, reflected by $-\text{SH}$ conformational changes, were significantly increased in untreated CD and UC patients compared with controls, indicating enhanced oxidative stress and a compromised systemic antioxidant defense ($p < 0.001$). Protein oxidation levels were approximately 4.5-fold higher in untreated CD and UC patients than in healthy controls ($p < 0.002$), with no significant difference observed between the two disease groups. These findings suggest an inadequate adaptive antioxidant response to excessive ROS production in IBD.

In contrast, protein oxidation levels were significantly reduced—by approximately 2.2-fold—in patients receiving immunosuppressive therapy with azathioprine and/or corticosteroids compared with untreated IBD patients ($p < 0.005$). Protein oxidation values in treated CD and UC patients did not differ significantly between groups, indicating a comparable therapeutic effect on oxidative protein damage.

Patients with IBD in remission exhibited plasma levels of GPx-1 activity, MDA concentration, ROS production, superoxide anion levels, and protein oxidation that were comparable to those of healthy controls; however, these differences did not reach statistical significance ($p > 0.056$).

Correlation analysis revealed significant positive associations among oxidative stress markers, particularly in patients with CD compared with UC. Significant correlations were observed between ROS production and $\bullet\text{O}_2^-$ activation ($r = 0.63$, $p < 0.05$), $\bullet\text{O}_2^-$ activation and protein oxidation ($r = 0.66$, $p < 0.05$), $\bullet\text{O}_2^-$ activation and MDA levels ($r = 0.71$, $p < 0.003$), and ROS production and GPx-1 activity ($r = 0.68$, $p < 0.002$).

DISCUSSION:

Excessive production of reactive oxygen species (ROS) and the subsequent accumulation of oxidative stress (OS) are increasingly recognized as key mechanisms contributing to immune dysregulation and inflammatory tissue injury in inflammatory bowel disease (IBD). Interactions among intestinal microbiota, dietary components, and activated immune cells promote excessive generation of pro-oxidants in the inflamed gut, thereby amplifying mucosal damage and perpetuating inflammation [1, 6, 19]. Recent evidence, including the findings of Xu et al. [19], highlights oxidative injury as a critical factor in both the initiation and progression of IBD. Similar redox disturbances observed in systemic inflammatory conditions, such as sepsis, further support the role of disrupted redox signaling, molecular damage, and impaired antioxidant defenses in disease pathophysiology. Collectively, these observations suggest that therapeutic strategies targeting ROS-mediated mechanisms may offer meaningful benefits in IBD management [19, 20].

Previous studies have demonstrated a reduction in antioxidant defense capacity within the intestinal mucosa of patients with active CD and UC [20, 21]. However, reported findings regarding glutathione peroxidase (GSH-Px/GPx) activity remain inconsistent, with some studies describing increased enzyme activity in both active and remission phases [21, 22], while others report no significant

differences between UC patients and healthy controls. These discrepancies may reflect disease heterogeneity and indicate the presence of distinct antioxidative mechanisms in CD and UC.

In the present study, serum GPx-1 levels differed significantly between patients with CD and UC and healthy controls ($p < 0.003$). Notably, antioxidant activity improved following therapeutic intervention, particularly in patients with CD ($p < 0.05$), suggesting a beneficial effect of standard anti-inflammatory treatments, including azathioprine, mesalazine, and corticosteroids. This observation is consistent with previous reports demonstrating that IBD therapy reduces ROS production and attenuates oxidative stress through suppression of inflammatory signaling and cellular proliferation [22, 23].

Azathioprine exerts its immunosuppressive effects through enzymatic conversion into active metabolites by phosphoribosyl transferase and thiopurine methyltransferase, leading to inhibition of purine synthesis and subsequent suppression of DNA and RNA replication [24]. This mechanism not only limits immune cell activation but may also reduce ROS generation and oxidative metabolic burden. In contrast, Thomas et al. [24] reported elevated plasma GPx levels in untreated CD patients, interpreted as a compensatory mucosal response to increased oxidative stress. Together, these findings suggest that effective anti-inflammatory therapy restores redox balance by reducing ROS-mediated damage and enhancing antioxidant defense capacity.

Overall, the present results support the concept that conventional IBD therapies contribute to disease control not only through immunomodulation but also via attenuation of oxidative stress. Strategies that reduce ROS production, limit cellular injury, and restore antioxidant enzyme activity—such as GPx-1—may therefore play a crucial role in promoting mucosal healing and improving clinical outcomes in patients with IBD [1, 2, 24].

Therapy-induced activation of the glutathione peroxidase (GPx) antioxidant defense system contributes to the reduction of hydroperoxides within the gastrointestinal tract. Intestinal inflammation in Crohn's disease (CD) is characterized by pronounced lipid peroxidation and increased levels of oxidized lipoproteins, particularly during active disease. Distinct oxidative mechanisms have been described in ulcerative colitis (UC) and CD: lipid peroxidation in UC has been linked to epithelial catalase (CAT) expression through hydrogen peroxide-mediated pathways, whereas in CD it is associated with mitochondrial superoxide dismutase (SOD) activity involving hydroxyl ($\bullet\text{OH}$) and superoxide ($\bullet\text{O}_2^-$) radicals [6, 8, 20].

In the present study, assessment of lipid peroxidation revealed a significant reduction in malondialdehyde (MDA) levels in both CD and UC patients treated with azathioprine, mesalazine, and corticosteroids compared with untreated patients ($p < 0.05$) (Figure 1B). Although the decrease in MDA levels was not significantly different between treated CD and UC patients, these findings suggest that pharmacological therapy effectively reduces lipid peroxide-mediated membrane damage. The combined thera-

peutic approach may synchronize the detoxification of hydrogen peroxide as well as hydroxyl and superoxide radicals by limiting inflammatory cell infiltration and promoting mucosal healing, particularly in CD.

Consistent with these observations, Tüzün et al. [21] reported lower MDA levels in CD patients during remission compared with healthy controls. Persistent oxidative damage, impaired regulation of antioxidant enzymes, and enhanced lipid peroxidation further underscore the central role of oxidative stress in CD pathogenesis [20, 25].

Emerging evidence also suggests that combining anti-inflammatory therapy with probiotics in IBD may enhance antioxidant enzyme activity and more effectively reduce ROS levels, thereby mitigating mucosal injury and restoring disrupted epithelial barrier integrity [26]. Consequently, further investigation of ROS-sensitive therapeutic strategies that augment endogenous antioxidant defenses and modulate ROS-driven inflammatory pathways is warranted.

Electron paramagnetic resonance (EPR) spectroscopy employing stable nitroxide spin probes enables quantitative assessment of oxidative stress (OS) and reactive oxygen species (ROS) accumulation in blood and tissues [27]. In the present study, ROS production, superoxide anion ($\bullet\text{O}_2^-$) activation, and protein oxidation were overall increased in patients with Crohn's disease (CD) and ulcerative colitis (UC), although these elevations did not reach statistical significance in some comparisons. These findings underscore the contribution of hydroxyl ($\bullet\text{OH}$) and superoxide radicals to oxidative stress-mediated cellular damage (Figure 2).

Following anti-inflammatory therapy, several oxidative stress markers demonstrated a statistically significant reduction. Specifically, ROS production (Figure 2A, $p < 0.005$), $\bullet\text{O}_2^-$ activation (Figure 2B, $p < 0.05$), and protein oxidation (Figure 2C, $p < 0.05$) decreased by approximately two-fold in both CD and UC patient groups. These results indicate that standard immunosuppressive therapy effectively attenuates systemic oxidative burden.

Consistent with these findings, Dudzińska et al. [25] reported a positive correlation between elevated ROS levels, lipid hydroperoxides (LOO \bullet /LOOH), and increased endogenous oxidative stress in patients with CD and UC. ROS overproduction appears to be a central factor in disease progression, oxidative tissue damage, and therapeutic responsiveness. Previous studies have also shown that both intestinal and peripheral T cells in CD and UC exhibit immunoregulatory abnormalities, while activated phagocytes infiltrating the intestinal mucosa represent the primary source of excessive ROS generation [28].

Moreover, Velayutham et al. [29] demonstrated approximately nine-fold higher serum $\bullet\text{O}_2^-$ activation compared with colonic tissue in UC, suggesting continuous release of tissue-derived oxidants into the systemic circulation. Similarly, Wang et al. [30] reported higher rates of oxidative stress induction during the active phase of CD and UC compared with remission.

In the present cohort, 17 of 21 patients (81.0%) were in the active phase of IBD at the time of evaluation. The

elevated inflammatory burden, together with combined immunosuppressive therapy (azathioprine, mesalazine, and corticosteroids), likely contributed to the observed two-fold regulation of ROS production, lipid peroxidation, and overall oxidative stress. The lack of statistically significant reductions in certain oxidative markers, particularly in CD patients, may be influenced by disease duration, timing of diagnosis, and the severity of inflammation and oxidative injury [30–33].

Finally, protein oxidation, reflecting conformational alterations of thiol (–SH) groups and formation of thiyl radicals (R–S•), demonstrated an approximately three-fold increase in systemic oxidative stress (OS) and excessive ROS production in patients with Crohn’s disease (CD) and ulcerative colitis (UC) compared with healthy controls. Reduced free thiol (R–SH) levels, enhanced protein conformational changes, and systemic OS strongly correlate with endoscopic disease activity in both CD and UC, even when compared with fecal calprotectin levels [20, 31]. Elevated protein oxidation in untreated CD and UC patients during the active disease phase was associated with increased inflammatory severity. In contrast, protein oxidation was markedly reduced in CD and UC patients receiving combined immunosuppressive therapy with azathioprine, mesalazine, and corticosteroids, supporting the role of systemic protein oxidation and thiol redox imbalance as key markers of oxidative stress and inflammation in IBD [32]. Recent evidence suggests that nitroxide-sensitive protein oxidation may serve as a sensitive biomarker for IBD activity, potentially outperforming C-reactive protein (CRP), which is influenced by confounding factors such as age and serum albumin levels [20, 30]. This marker may be particularly valuable for detecting moderate endoscopic activity and improving disease monitoring in CD and UC patients [20, 33].

Several limitations of the present study should be acknowledged. First, the relatively small sample size ($n = 21$) limits statistical power and may restrict the generalizability of the findings to broader pediatric IBD populations. Second, the cross-sectional, observational design precludes definitive conclusions regarding causal relationships between oxidative stress markers, disease activity, and therapeutic response. Third, although multiple OS parameters were assessed, measurements were confined to serum biomarkers and did not include tissue-specific analyses or direct assessment of intestinal mucosal redox status. Additionally, treatment regimens were not uniform across patients, and only a limited number received biological therapy, constraining comparative evaluation of therapy-specific oxidative stress responses.

Immunosuppressive agents, mesalazine, and biological therapies exert distinct mechanisms of action on intestinal mucosal inflammation in IBD. Given the limited number of patients receiving each specific pharmacological regimen in this cohort, definitive conclusions regarding the individual effects of these therapies on oxidative stress markers cannot be drawn. Therefore, larger, longitudinal, and prospective studies are warranted to clarify the

differential antioxidant and redox-modulating effects of specific therapeutic classes and to further validate oxidative stress biomarkers for clinical monitoring in IBD.

CONCLUSION:

This study underscores the pivotal role of oxidative stress (OS) and excessive reactive oxygen species (ROS) production in the pathophysiology of pediatric inflammatory bowel disease (IBD), including both Crohn’s disease (CD) and ulcerative colitis (UC). Treatment-naïve patients exhibited markedly elevated serum ROS levels, lipid peroxidation, and protein oxidation, reflecting a pronounced redox imbalance and impaired antioxidant defense. Notably, superoxide anion ($\bullet\text{O}_2^-$) levels were significantly increased during active disease, particularly in CD, and showed positive correlations with other OS markers, including malondialdehyde (MDA) and protein oxidation.

Serum GPx-1 activity was significantly reduced in untreated CD and UC patients but increased following immunosuppressive therapy with azathioprine and corticosteroids, indicating partial restoration of antioxidant capacity. Therapeutic intervention was associated with an approximately two-fold reduction in ROS production, lipid peroxidation, and protein oxidation, most prominently in UC patients, supporting the efficacy of standard anti-inflammatory regimens in attenuating oxidative damage and improving redox homeostasis. Furthermore, patients in remission demonstrated OS marker levels approaching those of healthy controls, reinforcing the link between effective disease control and normalization of oxidative balance.

Collectively, these findings suggest that OS-related biomarkers—particularly protein oxidation and $\bullet\text{O}_2^-$ generation—may serve as sensitive indicators of disease activity and therapeutic response in pediatric IBD. Given accumulating evidence that ROS, MDA, and protein oxidation correlate with inflammatory burden, incorporation of these markers into clinical monitoring as adjunct, noninvasive tools may enhance disease assessment. Serial measurement alongside established indices such as clinical activity scores, C-reactive protein, and fecal calprotectin could facilitate detection of subclinical inflammation, patient stratification based on oxidative stress status, and more precise monitoring of treatment efficacy. Ultimately, integrating oxidative stress biomarkers into routine follow-up may support earlier intervention, treatment optimization, and a more individualized approach to disease management. Future studies should further explore the therapeutic potential of antioxidant strategies in combination with conventional immunosuppressive treatments to mitigate ROS-mediated intestinal injury and promote mucosal healing.

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REFERENCES:

1. Iliopoulou L, Kollias G. Harnessing murine models of Crohn's disease ileitis to advance concepts of pathophysiology and treatment. *Mucosal Immunol.* 2022 Jan;15(1):10-26. [PubMed]
2. Abdel Hadi L, Di Vito C, Riboni L. Fostering Inflammatory Bowel Disease: Sphingolipid Strategies to Join Forces. *Mediators Inflamm.* 2016;2016:3827684. [PubMed]
3. Aviello G, Knaus UG. NADPH oxidases and ROS signaling in the gastrointestinal tract. *Mucosal Immunol.* 2018; 11:1011–23. [PubMed]
4. Xu J, Chu T, Yu T, Li N, Wang C, Li C, et al. Design of diselenide-bridged hyaluronic acid nano-antioxidant for efficient ROS scavenging to relieve colitis. *ACS Nano.* 2022; 16:13037-48. [Crossref]
5. Jakubczyk K, Dec K, Ka³duńska J, Kawczuga D, Kochman J, Janda K. Reactive oxygen species - sources, functions, oxidative damage. *Pol Merkur Lekarski.* 2020; 48:124-7. [PubMed]
7. Luceri C, Bigagli E, Agostiniani S, Giudici F, Zamboni D, Scaringi S, et al. Analysis of oxidative stress-related markers in crohn's disease patients at surgery and correlations with clinical findings. *Antioxidants.* 2019; 8:378. [PubMed]
8. Barrera G, Pizzimenti S, Daga M, Dianzani C, Arcaro A, Cetrangolo GP, et al. Lipid peroxidation-derived aldehydes, 4-hydroxynonenal and malondialdehyde in aging-related disorders. *Antioxidants.* 2018; 7:102. [PubMed]
9. Gêgotek A, Skrzydlewska E. Biological effect of protein modifications by lipid peroxidation products. *Chem Phys Lipids.* 2019; 221:46–52. [PubMed]
10. Miller CJ, Rose AL, Waite TD. Hydroxyl radical production by H₂O₂ - mediated oxidation of Fe(II) complexed by suwannee river fulvic acid under circumneutral freshwater conditions. *Environ Sci Technol.* 2013; 47:829–35. [PubMed]
11. Baschieri A, Jin Z, Amorati R. Hydroperoxyl radical (HOO•) as a reducing agent: unexpected synergy with antioxidants. A review. *Free Radic Res.* 2023; 57:115–29. [PubMed]
12. Sanders LM, Henderson CE, Hong MY, Barhouri R, Burghardt RC, Carroll RJ, et al. Pro-oxidant environment of the colon compared to the small intestine may contribute to greater cancer susceptibility. *Cancer Lett.* 2004; 208:155–61. [PubMed]
13. Harvey RF, Bradshaw JM. A simple index of Crohn's-disease activity. *Lancet.* 1980; 1:514. [PubMed]
14. Rachmilewitz D. Coated mesalazine (5-aminosalicylic acid) versus sulphasalazine in the treatment of active ulcerative colitis: a randomized trial. *British Medical Journal.* 1989; 298:82-6. [PubMed]
15. Buckell NA, Lennard-Jones JE, Hernandez MA, Kohn J, Riches PG, Wadsworth J. Measurements of serum proteins during attacks of ulcerative colitis as a guide to patient management. *Gut.* 1979;20: 22–7. [PubMed]
16. Shi H, Sui Y, Wang X, Luo Y, Ji L. Hydroxyl radical production and oxidative damage induced by cadmium and naphthalene in liver of *Carassius auratus*. *Comp. Biochem. Phys. Part C Toxicol. Pharmacol.* 2005; 140, 115–121. [PubMed]
17. Perrone S, Santacroce A, Longini M, Proietti F, Bazzini F, Buonocore, G. The free radical diseases of prematurity: From cellular mechanisms to bedside. *Oxidative Med Cell Longev.* 2018; 7483062. [PubMed]
18. Takeshita K, Saito K, Ueda JI, Anzai K, Ozawa T. Kinetic study on ESR signal decay of nitroxyl radicals, potent redox probes for in vivo ESR spectroscopy, caused by reactive oxygen species. *Biochim Biophys Acta.* 2002 Nov 14;1573(2):156-64. [PubMed]
19. Xu S, Li X, Zhang S, Qi C, Zhang Z, Ma R, et al. Oxidative stress gene expression, DNA methylation, and gut microbiota interaction trigger Crohn's disease: a multi-omics Mendelian randomization study. *BMC Med.* 2023; 21:179. [PubMed]
20. Muro P, Zhang L, Li S, Zhao Z, Jin T, Mao F, et al. The emerging role of oxidative stress in inflammatory bowel disease. *Front Endocrinol (Lausanne).* 2024 Jul 15;15:1390351. [PubMed]
21. Tüzün A, Erdil A, Ýnal V, Aydýn A, Bađcý S, Yepilova Z, et al. Oxidative stress and antioxidant capacity in patients with inflammatory bowel disease. *Clin biochem.* 2002; 35(7): 569-572. [PubMed]
22. Durak I, Yasa MH, Bektas A, Kacmaz M, Cimen MY, Ozturk HS. Mucosal antioxidant defense is not impaired in ulcerative colitis. *Hepato-gastroenterol.* 2000; 47:1015–7. [PubMed]
23. Tavassolifar MJ, Changaei M, Salehi Z, Ghasemi F, Javidan M, Nicknam MH, Pourmand MR. Redox imbalance in Crohn's disease patients is modulated by azathioprine. *Red Report.* 2021; 26(1), 80-84. [PubMed]
24. Thomas AG, Miller V, Shenkin A, Fell GS, Taylor F. Selenium and glutathione peroxidase status in paediatric health and gastrointestinal disease. *J Pediatr Gastroenterol Nutr* 1994; 19: 213–219. [PubMed]
25. Dudzińska E, Gryzinska M, Ognik K, Gil-Kulik P, Kocki J. Oxidative stress and effect of treatment on the oxidation product decomposition processes in IBD. *Oxid Med Cell Longevity.* 2018; 7918261. [PubMed]
26. Wan X, Zhang C, Lei P, Wang H, Chen R, Yang Q, Cheng Y, Wu W, Sun D, Hong X. Precision therapeutics for inflammatory bowel disease: advancing ROS-responsive nanoparticles for targeted and multifunctional drug delivery. *J Materials Chem.* 2025; 13(10): 3245-3269. [PubMed]
27. Georgieva E, Ananiev J, Yovchev Y, Arabadzhiev G, Abrashev H, Zaharieva V, et al. Stable Nitroxide as Diagnostic Tools for Monitoring of Oxidative Stress and Hypoalbuminemia in the Context of COVID-19. *Int J Mol Sci.* 2024 24 Jul.;25(15): 8045. [PubMed]
28. Petit C, Rozières A, Boschetti G, Viret C, Faure M, Nancey S, et al. Advances in Understanding Intestinal Homeostasis: Lessons from Inflammatory Bowel Disease and Monogenic Intestinal Disorder Pathogenesis. *Intern J Mol Sci.* 2025; 26(13), 6133. [PubMed]
29. Velayutham M, Kupec J, Khramtsov V and Rajendran V. Electron Paramagnetic Resonance (EPR) Spectroscopy to Measure Oxidative Stress (OS) in Ulcerative Colitis Mouse. *Physiol.* 2024; 39(S1), 1018.
30. Wang KY, Heikal OS, van

Rheenen PF, Touw DJ, Bourgonje AR, Mian P. Clinical and Biochemical Factors Associated with Infliximab Pharmacokinetics in Paediatric Patients with Inflammatory Bowel Disease. *J Clin Med*. 2025; 14(3):845. [\[PubMed\]](#)

31. Bourgonje AR, Gabriëls RY, de Borst MH, Bulthuis MLC, Faber KN, van Goor H. Serum free thiols are superior to fecal calprotectin in reflecting

endoscopic disease activity in inflammatory bowel disease. *Antioxidants*. 2019; 8:351. [\[PubMed\]](#)

32. Neubauer K, Kempinski R, Matusiewicz M, Bednarczyk Misa I, Krzystek-Korpacka M. Nonenzymatic serum antioxidant capacity in IBD and its association with the severity of bowel inflammation and corticosteroids treatment. *Med (B. Aires)*. 2019; 55:88.

[\[PubMed\]](#)

33. Bohra A, Batt N, Dutt K, Sluka P, Niewiadomski O, Vasudevan A, et al. Prospective Evaluation of Serum Free Thiols in Inflammatory Bowel Disease: A Candidate to Replace C-Reactive Protein for Disease Activity Assessment? *Inflamm Bowel Dis*. 2025 Feb 6;31(2):476-484. [\[PubMed\]](#)

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