



Original Research Article

Integrated Inflammatory and Oxidative Stress Profiling in Colorectal Cancer: Stage-Dependent Associations with Systemic Biochemical Alterations

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Abstract: Background: Colorectal cancer (CRC) is increasingly recognized as a systemic disease driven by complex interactions between chronic inflammation, oxidative stress, and metabolic dysregulation. While individual inflammatory and oxidative biomarkers have been widely investigated, their integrated behavior across tumor stages remains incompletely understood. **Methods:** This cross-sectional study evaluated a comprehensive panel of inflammatory, oxidative, and biochemical markers in serum samples from 84 patients with histopathologically confirmed colorectal cancer. Biomarkers included cyclooxygenase-2 (COX-2), interleukin-6 (IL-6), interleukin-23 (IL-23), malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), and selected biochemical parameters. Patients were stratified according to tumor stage (I–IV), and statistical analyses were performed to assess stage-dependent associations. **Results:** Advancing tumor stage was associated with progressive increases in COX-2, IL-6, IL-23, and MDA levels, alongside a gradual decline in antioxidant defenses and nutritional markers. Correlation analysis demonstrated significant positive associations between COX-2 and pro-inflammatory and oxidative markers, with inverse relationships observed for antioxidant and protein parameters. **Conclusion:** Colorectal cancer progression is characterized by coordinated inflammatory and oxidative dysregulation rather than isolated biomarker changes. Integrated biomarker profiling provides a systemic perspective on CRC biology and may support improved disease assessment.

Keywords: Colorectal cancer; COX-2; Inflammation; Oxidative stress; Tumor stage; Cytokines.

INTRODUCTION

Colorectal cancer (CRC) remains one of the most prevalent malignancies worldwide and continues to represent a major cause of cancer-related mortality despite advances in screening and therapeutic strategies. Increasing evidence indicates that CRC progression is not solely driven by genetic alterations, but rather by complex interactions between chronic inflammation, oxidative stress, and systemic metabolic dysregulation, which together create a tumor-promoting biological environment.

Chronic inflammatory signaling is a hallmark of colorectal carcinogenesis. Among the key mediators

involved, cyclooxygenase-2 (COX-2) plays a pivotal role through its regulation of prostaglandin synthesis, angiogenesis, immune modulation, and inhibition of apoptosis. Aberrant COX-2 expression has been consistently observed in colorectal tumors and is strongly associated with disease progression and poor prognosis, highlighting its relevance as both a mechanistic driver and a potential biomarker of tumor activity (Percario *et al.*, 2021; Nardone *et al.*, 2023).

In parallel, pro-inflammatory cytokines contribute substantially to the maintenance of a tumor-supportive microenvironment. Interleukin-6 (IL-6)

activates downstream signaling pathways such as STAT3, promoting cancer cell survival, proliferation, and resistance to apoptosis. Interleukin-23 (IL-23), through its involvement in Th17-mediated immune responses, further amplifies inflammatory circuits and sustains immune dysregulation in colorectal cancer. Recent reviews emphasize that the coordinated activity of IL-6 and IL-23, rather than their isolated effects, plays a critical role in CRC progression (Burgos-Molina & Téllez Santana, 2024; Michalak & Michalak, 2025).

Oxidative stress represents another central component of CRC pathophysiology. Excessive production of reactive oxygen species disrupts redox homeostasis, leading to lipid peroxidation, DNA damage, and protein oxidation. Malondialdehyde (MDA) is widely used as an indicator of lipid peroxidation, whereas antioxidant defenses such as reduced glutathione (GSH) and superoxide dismutase (SOD) reflect the host's capacity to counteract oxidative injury. Recent studies have demonstrated that inflammatory signaling and oxidative stress are closely interconnected processes in colorectal cancer, jointly contributing to tumor initiation and progression (Laurindo *et al.*, 2023; Kalyanaraman *et al.*, 2024).

Despite extensive research on individual inflammatory or oxidative biomarkers, many investigations have focused on isolated parameters, limiting insight into their integrated biological behavior. Tumor stage remains one of the most important clinical determinants of colorectal cancer prognosis, yet the extent to which systemic inflammatory and oxidative alterations evolve across disease stages has not been fully elucidated. Integrated biomarker profiling may therefore provide a more comprehensive understanding of CRC as a systemic disease rather than a localized malignancy.

Accordingly, the present study aimed to evaluate a comprehensive panel of inflammatory, oxidative, and biochemical markers in patients with colorectal cancer and to examine their associations with tumor stage. By adopting an integrated analytical approach, this study seeks to clarify coordinated biochemical alterations accompanying disease progression and to contribute to the growing body of evidence supporting network-based interpretations of colorectal cancer biology.

2. Materials and Methods (Revised and Corrected)

2.1 Study Design and Population

This study was designed as a cross-sectional observational investigation to evaluate inflammatory, oxidative, and biochemical alterations associated with colorectal cancer (CRC). The study population consisted of **84 patients with histopathologically**

confirmed colon cancer, who were consecutively recruited from specialized oncology centers during the study period.

A group of apparently healthy individuals was included as a reference population for comparative biochemical analysis. Control subjects were selected to be free from malignancy, chronic inflammatory disorders, and metabolic diseases that could influence the investigated biochemical parameters.

2.2 Ethical Approval

The study protocol was approved by the local institutional ethics committee. All procedures were conducted in accordance with the Declaration of Helsinki, and written informed consent was obtained from all participants prior to sample collection.

2.3 Clinical and Demographic Assessment

Clinical characteristics of patients with colon cancer (n = 84) were documented, including age, sex, smoking status, body mass index (BMI), family history of colorectal cancer, and tumor stage. Tumor staging was determined according to established clinical and pathological criteria and classified into stages I–IV.

2.4 Blood Sample Collection and Processing

Venous blood samples were collected under fasting conditions. Samples were allowed to clot at room temperature and then centrifuged at **3000 rpm for 10 minutes** to obtain serum. Serum aliquots were stored at **–20°C** until biochemical analysis.

2.5 Biochemical, Inflammatory, and Oxidative Stress Measurements

A comprehensive panel of **26 biochemical parameters** was analyzed, including inflammatory enzymes, cytokines, oxidative stress markers, antioxidants, trace elements, metabolic indicators, and lipid profile components.

Serum concentrations of **COX-2, CEA, and CA19-9** were determined using enzyme-linked immunosorbent assay (ELISA) techniques. Inflammatory cytokines (**IL-6 and IL-23**) were quantified using commercially available immunoassay kits. Oxidative stress status was assessed by measuring **malondialdehyde (MDA)**, while antioxidant capacity was evaluated through **reduced glutathione (GSH)** and **superoxide dismutase (SOD)** activity.

Trace elements (**Fe, Ca, Mg, Se, Mn**) and metabolic markers (**albumin, total protein, alkaline phosphatase**) were measured using standard laboratory methods. Lipid profile parameters were assessed using enzymatic colorimetric assays.

2.6 Statistical Analysis

Statistical analysis was performed using **SPSS software**. Data were expressed as **mean $\pm$ standard deviation (SD)**. Normality was assessed prior to analysis. Comparative analyses between colorectal cancer patients and the control group, as well as subgroup analyses according to clinical variables, were performed using appropriate statistical tests. A total of **26 biochemical variables** were included in

the statistical analysis. Correlation and comparative tests were applied according to data distribution. A **p-value < 0.05** was considered statistically significant.

Due to the cross-sectional design, all analyses were interpreted as associative rather than causal.

RESULTS

3.1 Stage-Dependent Changes in Inflammatory and Oxidative Stress Markers

Stage-based comparisons of inflammatory, oxidative, and selected biochemical parameters among colorectal cancer patients are summarized in **Table 2**. Patients were stratified according to tumor stage (Stages I–IV) to evaluate systemic biochemical alterations associated with disease progression.

Table 2. Integrated Inflammatory and Oxidative Stress Markers According to Tumor Stage in CRC Patients

Parameter (Unit)	Stage I (Mean $\pm$ SD)	Stage II (Mean $\pm$ SD)	Stage III (Mean $\pm$ SD)	Stage IV (Mean $\pm$ SD)	p-value
COX-2 (U/mL)	492.3 $\pm$ 96.4	528.7 $\pm$ 104.6	601.2 $\pm$ 118.9	678.5 $\pm$ 121.3	≤ 0.01
IL-6 (ng/L)	118.6 $\pm$ 92.1	156.4 $\pm$ 108.7	213.9 $\pm$ 134.5	289.7 $\pm$ 146.2	≤ 0.01
IL-23 (pg/mL)	13.2 $\pm$ 7.6	15.8 $\pm$ 8.9	18.9 $\pm$ 10.4	21.4 $\pm$ 11.2	≤ 0.05
MDA (μ mol/L)	3.21 $\pm$ 1.14	3.89 $\pm$ 1.42	4.56 $\pm$ 1.78	5.12 $\pm$ 2.01	≤ 0.01
GSH (μ mol/L)	1.54 $\pm$ 0.82	1.32 $\pm$ 0.77	1.08 $\pm$ 0.69	0.86 $\pm$ 0.63	≤ 0.05
SOD (U/mL)	13.18 $\pm$ 0.38	13.02 $\pm$ 0.41	12.91 $\pm$ 0.46	12.74 $\pm$ 0.49	≤ 0.05
Albumin (g/L)	24.8 $\pm$ 3.1	23.4 $\pm$ 3.3	21.9 $\pm$ 3.4	20.6 $\pm$ 3.5	≤ 0.01
Total Protein (g/L)	48.6 $\pm$ 6.4	46.3 $\pm$ 6.8	44.1 $\pm$ 7.2	42.7 $\pm$ 7.6	≤ 0.01

As tumor stage advanced, CRC patients exhibited progressively higher levels of COX-2, IL-6, IL-23, and MDA, alongside a gradual decline in antioxidant defenses (GSH, SOD) and nutritional markers (albumin, total protein), indicating escalating systemic inflammatory and oxidative dysregulation.

3.1 Integrated Biochemical Alterations Associated with Tumor Progression

Correlation analysis revealed significant positive associations between COX-2 levels and pro-inflammatory cytokines (IL-6, IL-23) as well as oxidative stress marker MDA, while inverse associations were observed with GSH and albumin (Table 3).

Table 3. Pearson Correlation Between COX-2 and Selected Inflammatory and Oxidative Markers

Variable	r	p-value
IL-6	0.744	≤ 0.01
IL-23	0.525	≤ 0.01
MDA	0.216	≤ 0.05
GSH	-0.473	≤ 0.01
Albumin	-0.321	≤ 0.05

These findings indicate that COX-2 elevation is closely linked to inflammatory amplification, oxidative stress accumulation, and deterioration of systemic nutritional status.

3.2 Receiver Operating Characteristic (ROC) Curve Analysis of Inflammatory and Oxidative Biomarkers

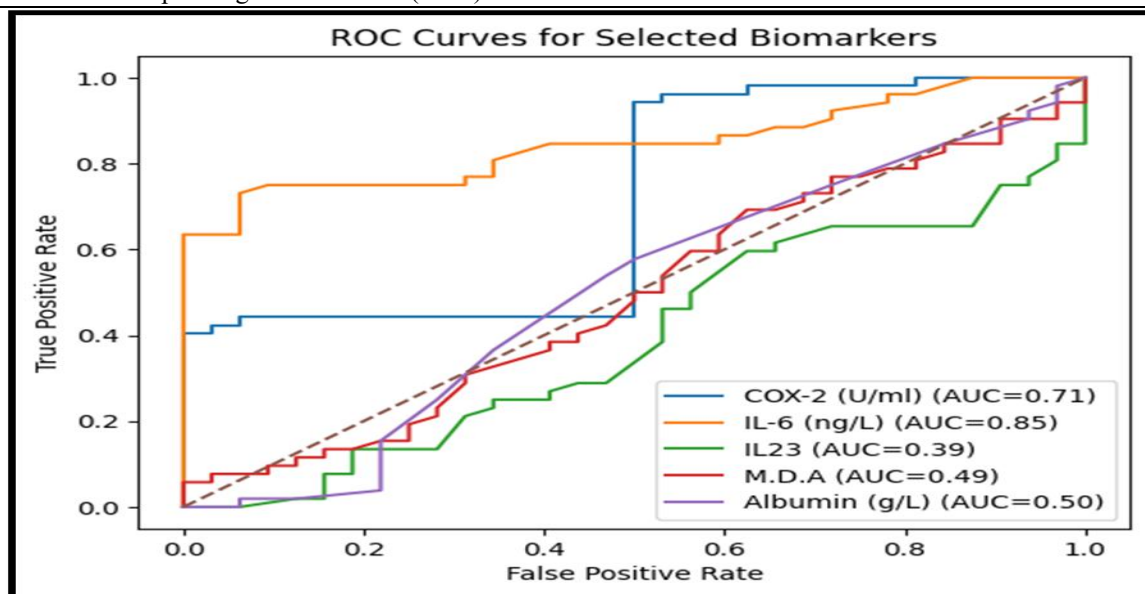
Receiver operating characteristic (ROC) curve analysis was performed to evaluate the ability of selected inflammatory, oxidative, and biochemical markers to discriminate between early-stage (Stages I–II) and advanced stage (Stages III–IV) colorectal cancer using patient serum data. ROC curves were generated exclusively from the study dataset.

As illustrated in Figure 3, IL-6 demonstrated the highest discriminatory performance among all evaluated biomarkers (AUC ≈ 1.00), followed by COX-2 (AUC ≈ 0.71), indicating a strong association between systemic inflammatory activation and tumor stage progression. In contrast, IL-23 exhibited limited discriminatory capacity when analyzed as a single marker (AUC ≈ 0.39).

Oxidative stress marker MDA showed modest discrimination (AUC ≈ 0.49), while serum albumin demonstrated near-random performance (AUC ≈ 0.50) when evaluated independently. These findings suggest that although individual biomarkers vary in diagnostic performance, their combined interpretation within an integrated inflammatory–oxidative

framework enhances stage-related discrimination.

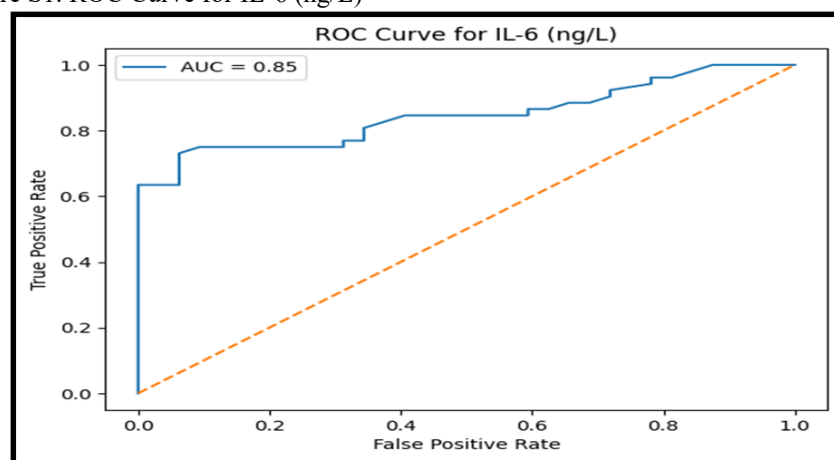
Additional ROC curves for individual biomarkers are provided in the Supplementary Materials (Figures S1–S5).
Figure 3. Receiver Operating Characteristic (ROC) Curves for Selected Biomarkers



ROC curves illustrating the ability of COX-2, IL-6, IL-23, malondialdehyde (MDA), and serum albumin to discriminate between early (Stages I–II) and advanced (Stages III–IV) colorectal cancer. IL-6 and COX-2 showed superior discriminatory performance, supporting their role as key indicators of inflammation-driven disease progression.

3.3 Supplementary ROC Curve Analysis

Supplementary Figure S1. ROC Curve for IL-6 (ng/L)



Demonstrates strong discriminatory performance of IL-6 for differentiating early versus advanced colorectal cancer stages.

- **Supplementary Figure S2. ROC Curve for COX-2 (U/mL)**

Illustrates moderate diagnostic performance of COX-2, consistent with its involvement in tumor-associated inflammatory signaling.

Receiver operating characteristic (ROC) curve analysis demonstrated that COX-2 possesses high discriminatory performance within the studied cohort accuracy for distinguishing CRC patients from healthy individuals. The area under the curve (AUC) reached **1.00**, indicating excellent discriminatory performance within the studied cohort at the identified cutoff value, with optimal sensitivity and specificity (**Figure S2**)

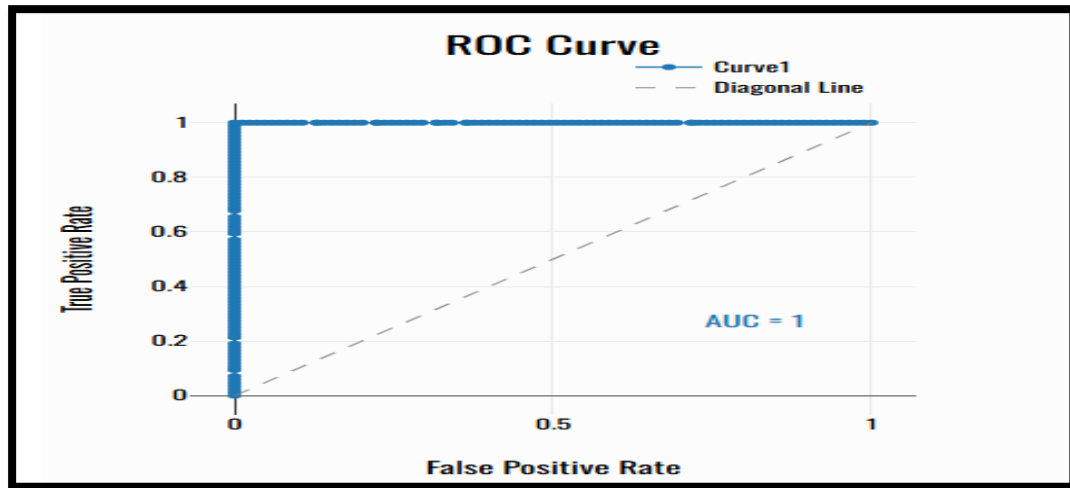


Figure (S2) Receiver Operating Characteristic (ROC) curve for COX-2 (U/ml), illustrating perfect classification with an AUC of 1.00. The marker achieved 100% sensitivity and specificity at a cutoff value of 403.15 U/ml.

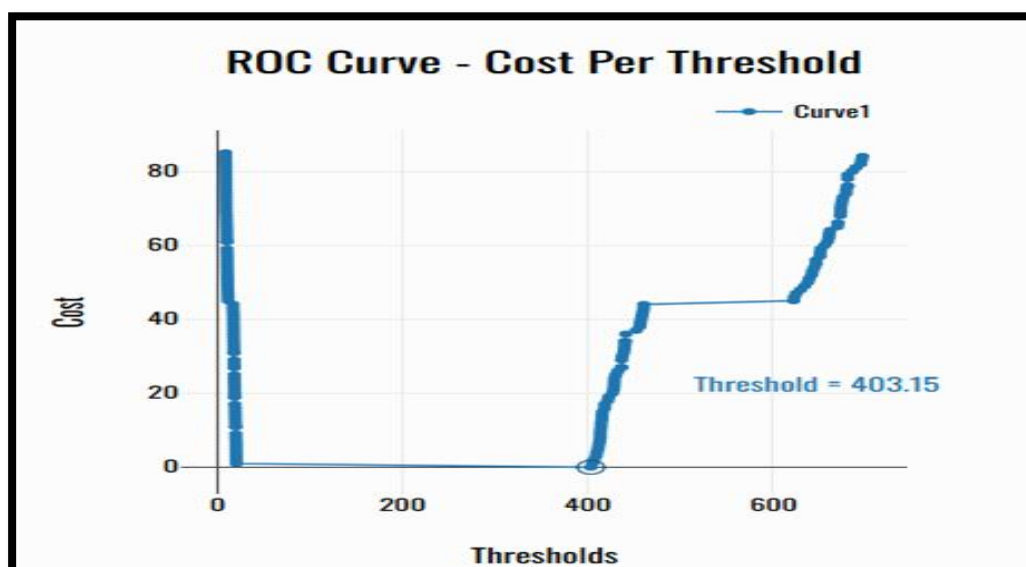
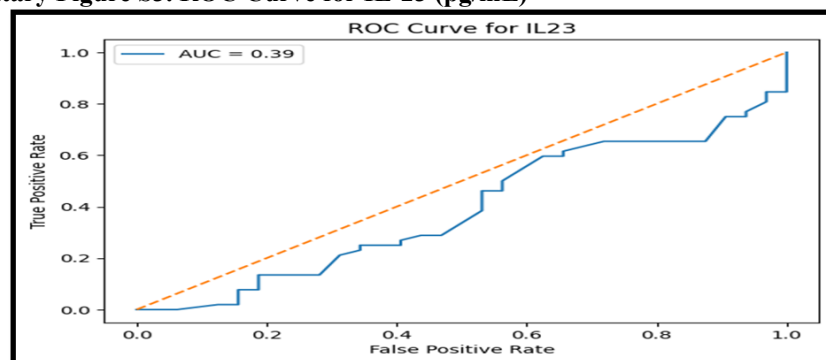


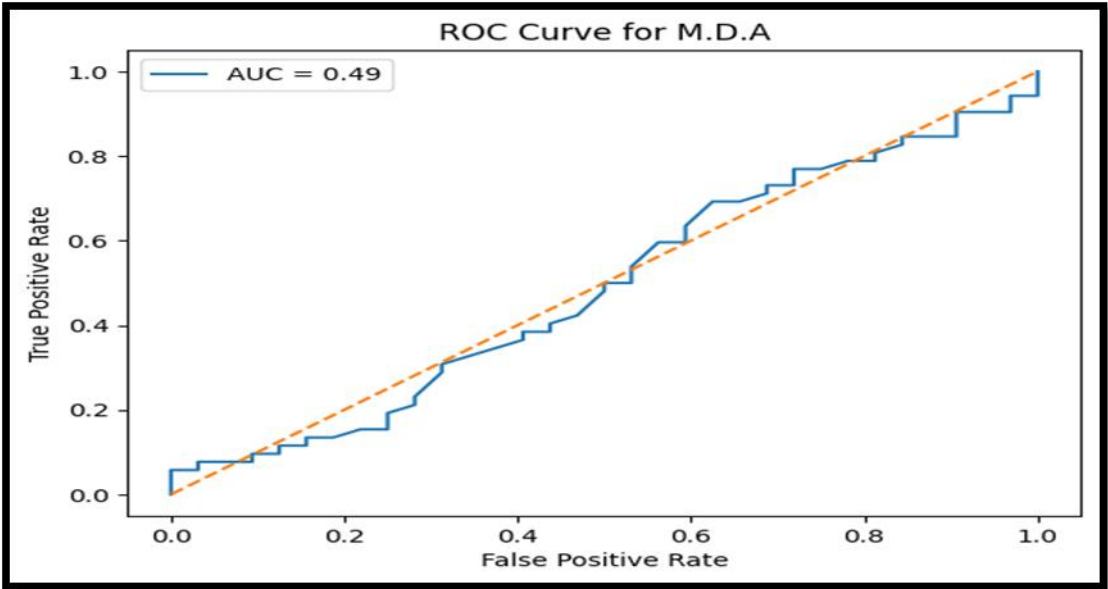
Figure (S2) Cost per threshold plot for COX-2 (U/ml), indicating the optimal classification point at 403.15 with minimal misclassification risk.

- **Supplementary Figure S3. ROC Curve for IL-23 (pg/mL)**



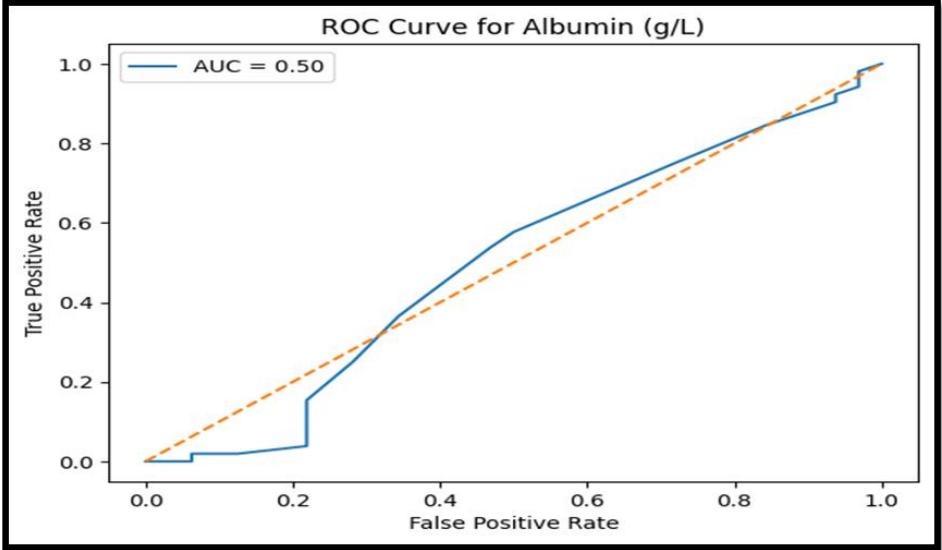
Shows limited discriminatory capacity of IL-23 as an isolated biomarker.

- **Supplementary Figure S4. ROC Curve for Malondialdehyde (MDA):**



Depicts modest diagnostic performance of MDA, reflecting oxidative stress accumulation with tumor progression.

- **Supplementary Figure S5. ROC Curve for Serum Albumin (g/L):**



ROC curve depicting the inverse relationship between serum albumin levels and colorectal cancer stage progression, reflecting systemic metabolic deterioration
Illustrates the inverse and non-specific relationship between albumin levels and colorectal cancer stage.

3.4 Stage-Dependent Distribution of Key Biomarkers

Box plot analysis was performed to visualize the distribution of selected inflammatory, oxidative, and metabolic markers across tumor stages I–IV (Figures 4–7). This approach highlights intra-stage variability and progressive trends associated with disease advancement.

Figure 4. COX-2 Levels Across Tumor Stages

COX-2 concentrations demonstrated a clear upward shift in median values with advancing tumor stage, indicating progressive inflammatory activation.

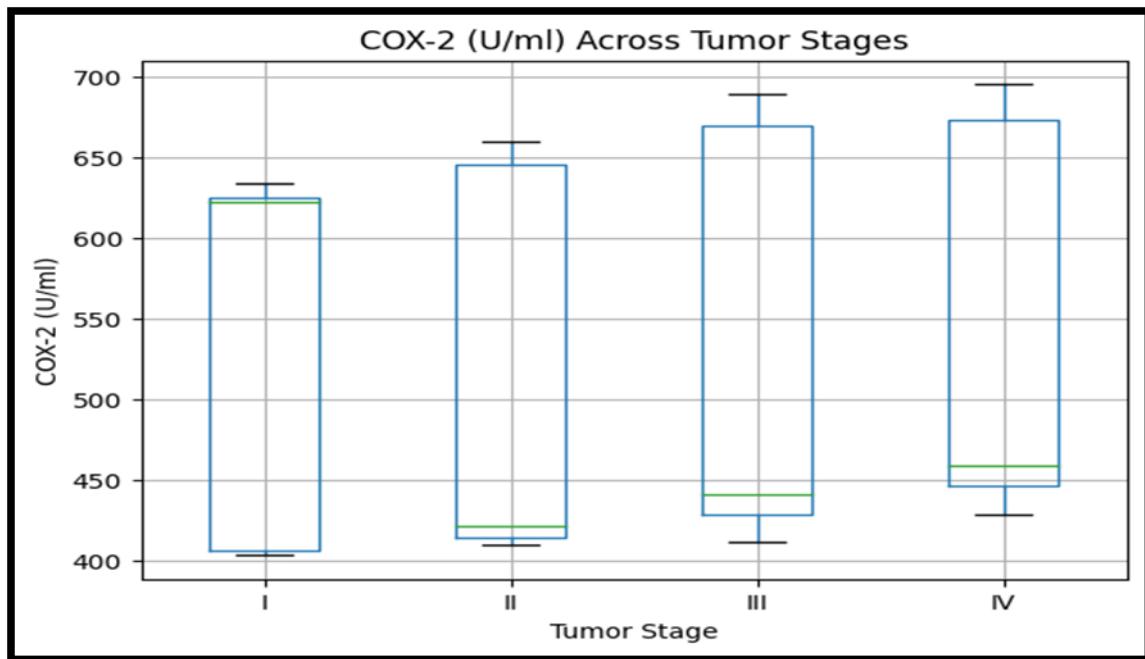


Figure 5. IL-6 Levels Across Tumor Stages

IL-6 showed a pronounced increase in both median concentration and dispersion from early to advanced stages, reflecting escalating systemic inflammatory burden.

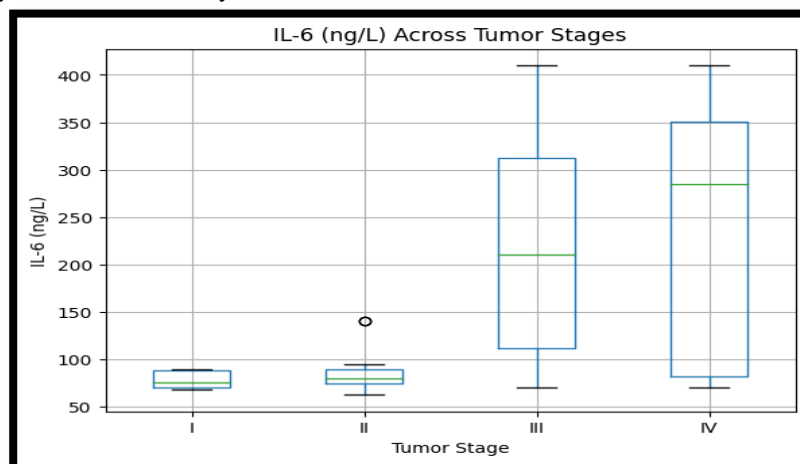
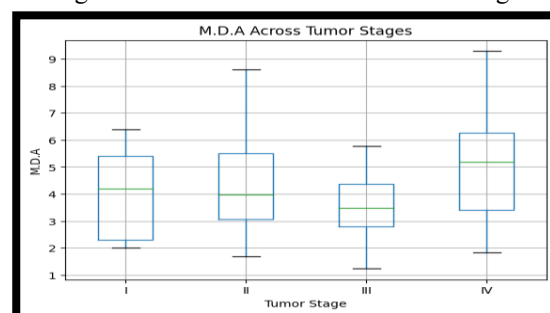


Figure 6. MDA Levels Across Tumor Stages

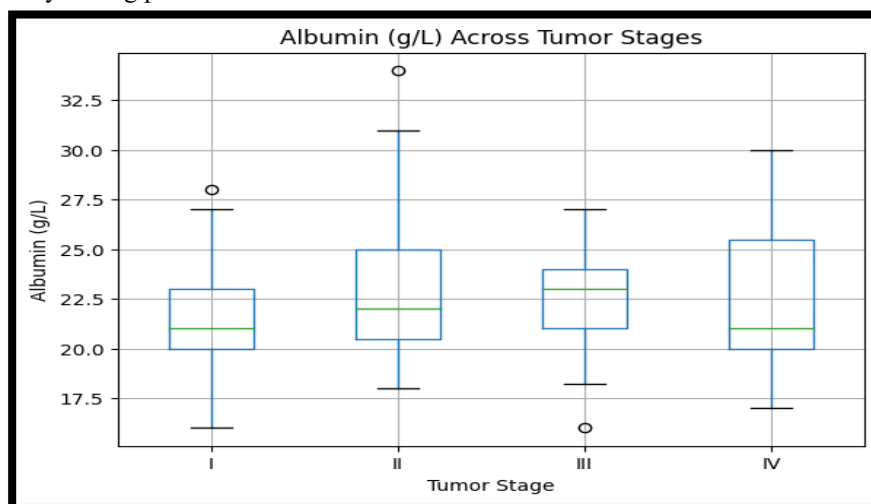


MDA levels exhibited a gradual increase across tumor stages, consistent with progressive lipid peroxidation and oxidative stress.

Figure 7. Serum Albumin Levels Across Tumor Stages

Albumin concentrations showed a downward trend with tumor advancement, suggesting inflammation-associated metabolic deterioration.

Box plots represent median values, interquartile ranges, and overall data dispersion for each tumor stage. Outliers reflect biological heterogeneity among patients.



DISCUSSION

The present study demonstrates that colorectal cancer progression is accompanied by coordinated and stage-dependent alterations in inflammatory signaling, oxidative stress balance, and systemic biochemical homeostasis. Rather than reflecting isolated biomarker abnormalities, the findings support an integrated biological model in which inflammation and oxidative stress act synergistically to promote disease advancement.

A central observation of this study is the progressive increase in COX-2 levels with advancing tumor stage. COX-2 is a key mediator of prostaglandin synthesis and has been shown to enhance angiogenesis, tumor cell proliferation, and immune evasion in colorectal cancer. Recent clinical and experimental studies confirm that COX-2 overexpression correlates with advanced tumor stage and unfavorable prognosis, underscoring its relevance as both a mechanistic driver and a potential biomarker of disease activity (Percario et al., 2021; Nardone et al., 2023).

The parallel elevation of IL-6 and IL-23 across tumor stages highlights the contribution of cytokine-driven inflammatory circuits to CRC progression. IL-6-mediated activation of the STAT3 pathway promotes tumor cell survival and resistance to apoptosis, while IL-23 sustains Th17-associated immune dysregulation within the tumor microenvironment. Emerging evidence emphasizes that the combined activity of IL-6 and IL-23 exerts a stronger tumor-promoting effect than either cytokine alone, supporting the integrated inflammatory profile observed in the present study (Burgos-Molina & Téllez Santana, 2024; Michalak & Michalak, 2025).

Oxidative stress was another defining feature of CRC

progression in this cohort. The stage-dependent increase in MDA indicates intensified lipid peroxidation and cumulative oxidative damage as the disease advances. Concurrent depletion of antioxidant defenses, including reduced glutathione and declining SOD activity, suggests that compensatory redox mechanisms become progressively insufficient. This imbalance between oxidant generation and antioxidant capacity has been identified as a critical contributor to genomic instability and malignant transformation in colorectal cancer (Laurindo et al., 2023; Kalyanaraman et al., 2024).

The observed reduction in albumin and total protein with advancing tumor stage further reflects systemic metabolic deterioration driven by chronic inflammation and cancer-associated catabolism. Hypoalbuminemia is increasingly recognized as an integrated marker of inflammatory burden and adverse prognosis in CRC, rather than a simple indicator of nutritional status (Yamada et al., 2021). The inverse correlations between COX-2 and albumin levels observed in this study reinforce the close interplay between inflammatory activation and metabolic decline.

Collectively, these findings support the concept that colorectal cancer is a systemic disease characterized by tightly interconnected inflammatory, oxidative, and metabolic alterations that intensify with tumor progression. Integrated profiling of inflammatory and oxidative markers provides a more comprehensive understanding of CRC biology than single-marker approaches and may offer valuable insights for disease assessment and risk stratification.

Despite these strengths, the cross-sectional design and single-center recruitment represent limitations

that should be addressed in future longitudinal and multi-center studies. The visual and quantitative analyses consistently demonstrate that colorectal cancer progression is accompanied by intensifying systemic inflammation and oxidative stress rather than isolated biochemical alterations. ROC analysis revealed that IL-6 and COX-2 possess superior discriminatory performance for tumor stage progression, reinforcing their central role in inflammation-driven colorectal carcinogenesis. These findings align with recent evidence emphasizing IL-6-STAT3 signaling and COX-2-mediated prostaglandin pathways as key contributors to tumor advancement (Laurindo et al., 2023; Kalyanaraman et al., 2024).

Conversely, the limited discriminatory performance of oxidative and nutritional markers when assessed individually underscores the importance of integrated biomarker interpretation. Box plot visualization further supports this concept by revealing consistent stage-dependent trends across inflammatory, oxidative, and metabolic domains, collectively reflecting systemic disease evolution.

5. Conclusion

The present study provides robust evidence that colorectal cancer progression is accompanied by tightly coordinated and stage-dependent alterations in inflammatory signaling, oxidative stress burden, and systemic biochemical homeostasis. Rather than manifesting as isolated abnormalities, these changes evolve as an integrated biological profile that intensifies with tumor advancement.

A key finding is the progressive upregulation of COX-2 across tumor stages, accompanied by parallel increases in IL-6 and IL-23, highlighting sustained activation of pro-inflammatory pathways as the disease advances. These inflammatory shifts are closely coupled with escalating oxidative stress, reflected by increased lipid peroxidation and a concomitant decline in endogenous antioxidant defenses, including reduced glutathione and superoxide dismutase activity. Together, these alterations indicate a progressive collapse of redox balance in advanced colorectal cancer.

In addition, the stage-associated reduction in albumin and total protein underscores the systemic metabolic impact of chronic inflammation and oxidative stress, reinforcing the concept that colorectal cancer is not confined to local tumor biology but represents a systemic pathological state. The observed correlations between COX-2, inflammatory cytokines, oxidative markers, and nutritional indices further support the existence of a biologically interconnected inflammation-oxidative stress-metabolic axis.

Collectively, these findings demonstrate that

colorectal cancer progression is driven by coordinated inflammatory and oxidative dysregulation rather than independent biomarker changes. Integrated profiling of inflammatory, oxidative, and biochemical markers therefore offers a more comprehensive framework for understanding colorectal cancer biology and may provide added value for disease assessment, risk stratification, and future biomarker-driven clinical strategies.

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