RESEARCH ARTICLE

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Study the Impact of NADPH oxidase1,TNF-alpha and Cyclooxygenase-2 On Colon Cancer Patients

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Abstract

Background: Colon cancer is a major health concern globally, being one of the most commonly diagnosed cancers. Inflammation and oxidative stress are critical factors contributing to its development and progression. Understanding the roles of key molecules such as NOX1, TNF-α, and COX-2 in these processes may provide insights into potential biomarkers for early detection and targeted therapy. **Objective:** This study aims to investigate the roles of NADPH oxidase 1 (NOX1), Tumor Necrosis Factor-alpha (TNF-α), and Cyclooxygenase-2 (COX-2) in colon cancer progression and their possible implications in the disease context. **Methods:** A comparative analysis was conducted involving 40 colon cancer patients (15 males, 25 females; aged 20-78 years) and 40 healthy controls (17 males, 23 females; aged 21-75 years). Blood samples were collected from participants between October 10, 2023, and February 18, 2024. The levels of NOX1, TNF-α, and COX-2 were measured using the enzyme-linked immunosorbent assay (ELISA) method. **Results:** The study found significantly elevated levels of TNF-α in colon cancer patients (100.9-454.3 ng/L) compared to healthy controls (24.85-216.9 ng/L). Additionally, COX-2 levels were markedly higher in patients (239.4-690.53 units/L) than in controls (23.78-115.5 units/L). NOX1 levels were also elevated in cancer patients (4.0-14.92) relative to healthy subjects (0.89-9.39). These findings suggest an association between increased levels of TNF-α, COX-2, and NOX1 with a higher risk of colon cancer. **Conclusion:** The findings suggest that elevated levels of TNF-α, COX-2, and NOX1 may be associated with the progression of colon cancer, indicating their potential involvement in the disease. Specifically, higher levels of TNF-α and COX-2 could be linked to increased cancer cell proliferation and metastasis, while elevated NOX1 levels might be related to oxidative stress and cellular transformation in colon cancer patients.

Keywords: Colon cancer- TNF-α- COX-2- NADPH oxidase 1- biomarkers.

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Introduction

Colon cancer, inclusive of rectal cancer under the term colorectal cancer, remains a critical health issue and ranks among the most prevalent cancers globally. This malignancy originates from polyps in the colonic mucosa, which can undergo malignant transformation if not excised [1]. The progression of the disease allows it to infiltrate the colon wall and metastasize to distant organs via the lymphatic system or bloodstream. Colon cancer significantly impacts global health, being the second leading cause of cancer-related mortality worldwide. In 2020, the incidence of colorectal cancer surpassed 1.9 million new cases, with approximately 930,000 associated deaths. Projections for 2040 suggest a considerable increase, with expected new cases reaching 3.2 million and deaths rising to 1.6 million, highlighting an urgent need for enhanced preventive measures and treatments [2].

Inflammation and oxidative stress are pivotal factors in the onset and progression of colorectal cancer. Chronic inflammation, frequently observed in conditions such as inflammatory bowel disease, compromises the integrity of the intestinal mucosa, rendering it susceptible to damage from pathogens and carcinogens [3]. This damage initiates cycles of regeneration and repair, which can lead to dysplastic alterations in epithelial cells, culminating in cancer development. Oxidative stress, defined by an imbalance between the generation of reactive oxygen species (ROS) and the body's antioxidant defenses, contributes to carcinogenesis through DNA damage, lipid peroxidation, and protein modification. Elevated ROS levels promote mutations, enhance inflammatory pathways such as NF-κB, and modify the tumor microenvironment, thereby fostering cancer cell survival and proliferation [4, 5].

Moreover, inflammation and oxidative stress not only function as risk factors but also serve as potential biomarkers for disease progression and prognosis. Numerous studies have highlighted the relationships between inflammatory markers, oxidative stress indicators, and clinical outcomes in colorectal cancer (CRC). The interplay between inflammation and oxidative stress

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sustains a cycle of damage and repair, creating an environment conducive to colorectal cancer development [6, 7].

NADPH oxidases are components of a complex group of proteins that produce superoxide radicals, typically membrane-bound and activated by cytosolic factors through signaling events and receptors. Their primary role is in host defense, protecting against microbial diseases [8]. Primarily expressed in phagocytic blood cells, NADPH oxidases generate ROS, which, while crucial for immunity and cell growth, can lead to oxidative stress when overproduced. NOX1, a predominant isoform in colon cells, shows increased expression in many tumors, suggesting its involvement in carcinogenesis [9]. The NOX family of NADPH oxidases plays diverse roles in gene expression, cellular signaling, and immune defense. Deficiency in NOX can lead to compromised immunity, while excess ROS production can result in cellular stress and various diseases, including cancer. NOX1, in particular, is highly expressed in the colon, prostate, uterus, and vascular cells, and its upregulation is associated with several types of human tumors, highlighting its importance in tumorigenesis [10].

In addition to the role of NADPH oxidase, tumor necrosis factor-alpha (TNF-α) is crucial for immunity and inflammation, stimulating responses such as programmed cell death and necrosis. TNF-α has the potential to generate superoxide radicals in vascular and phagocytic cells through the activation of NADPH oxidase NOX2/gp91 [11]. While TNF-α has anti-cancer properties, its use in chemotherapy is limited due to systemic toxicity, including risks of systemic shock and widespread inflammatory responses. Recent studies have focused on TNF-α mutants with higher tumor-selective cytotoxicity and lower systemic toxicity, potentially enhancing anti-cancer therapies. For instance, TNF mutants with higher affinities to TNFR-1 and TNFR-2 have shown increased bioactivity and longer plasma half-lives, significantly improving their therapeutic windows [12]. TNF-α can also be used as an adjuvant to enhance the efficacy of chemotherapy agents, sensitize carcinomas to anti-EGFR therapy, and overcome resistance to EGFR tyrosine kinase inhibitors in non-small-cell lung cancer cells [13].

Another significant factor in colon cancer is Cyclooxygenase-2 (COX-2), with overexpression observed in many colorectal cancer patients. Genetic changes, lifestyle factors such as alcohol consumption, smoking, and dietary habits contribute to the high expression of COX-2. Chronic infections and inflammatory conditions also play roles in its upregulation [14]. Numerous studies have reported elevated levels of COX-2 mRNA and protein in colorectal adenocarcinomas compared to adjacent normal tissues. The overexpression of COX-2 is strongly associated with tumor growth, angiogenesis, and resistance to apoptosis, making it a potential target for therapeutic intervention. COX-2 inhibitors are being explored for their potential to reduce the risk of colorectal cancer and improve patient outcomes [15].

Given the critical roles of NOX1, COX-2, and TNF-α in oxidative stress and inflammation in colorectal cancer, these molecules may serve as potential biomarkers for predicting disease status. Therefore, the aim of this study is to measure the levels of NOX1, COX-2, and TNF-α in colon cancer patients compared to healthy controls, to better understand their roles in the disease and identify potential biomarkers for early detection and prognosis.

Materials and Methods

Study Population: This study was conducted in Al-Diwaniyah Governorate, Iraq, involving 40 colon cancer patients at various stages of the disease (from stage I to stage IV) and 40 healthy controls. Participants were selected during the period from October 10, 2023, to February 18, 2024. The age range of the participants was 20 to 78 years. Age was a key criterion in the selection process, and patients were undergoing treatment during the study period.

Methods: A 4 ml blood sample was collected from each participant and placed in a gel tube. The blood samples were centrifuged at 3600 rpm for 10-15 minutes at 4°C to separate the serum. The serum was subsequently divided into three aliquots using Eppendorf tubes, which were stored at -20°C for biochemical analysis. The levels of COX-2, NOX1, and TNF-α in the serum were quantified using the sandwich ELISA method. In this assay, specific antibodies were used to capture the target proteins. After binding, the samples were processed, and the optical density (OD) was read at 450 nm with a reference filter at 620 nm. Quantification was performed by comparing the OD values to a standard curve generated from known concentrations of the target proteins.

Inclusion Criteria: Participants diagnosed clinically with colon cancer by a specialist physician and having a documented history of chronic disease related to colon cancer within the past months were included in the study.

Exclusion Criteria: Patients with systemic or local diseases other than colon cancer, as well as those with a history of other malignant tumors, were excluded from the study. Statistical Analysis: Data were gathered, analyzed, and presented using Microsoft Office Excel 2013 and GraphPad Prism 9.2.0. Categorical data were expressed in numbers, while numerical data were presented as mean ± Standard Error of the Mean (SEM). An unpaired t-test and one-way ANOVA were performed to compare mean values between different groups for normally distributed variables. Qualitative data were analyzed using chi-square tests. Bivariate correlation was assessed using Pearson's correlation coefficient. A P-value of less than 0.05 was considered statistically significant.

Results

Demographic Characteristics

The demographic characteristics of the control group and the colon cancer patient group are compared in Table 1. The analysis revealed no significant variation in the gender distribution between the control group and the colon cancer patient group ($p = 0.98$). However, the proportion of females in the colon cancer patient group was higher than that of males, with 25 females (62.5%) and 15 males (37.5%). This suggests a higher susceptibility to

Table 1. Comparison of Demographic Characteristics between the Control Group and Patients in the Colon Cancer Group

Characteristic	Control		Patient with colon cancer		P value
Gender	Male	Female	Male	Female	0.98
	n (17)	n (23)	n (15)	n (25)	ns
	42.50%	57.50%	37.50%	62.50%	
Age (years)					
Mean ±SEM	20 - 72		20 - 72		0.0692
Range	42.10 ± 2.072		47.85 ± 2.333		ns
Body mass index BMI (kg/m^2)					
Mean ±SEM	21.56 - 33.53		23.24 - 36.84		0.0084
Range	26.94 ± 0.5472		29.17 ± 0.6181		**

colon cancer among females. Regarding age, there was no significant difference between the control group and the colon cancer patient group (p = 0.0692). The mean age of the control group was 42.10 ± 2.072 years, while the mean age of the patient group was 47.85 ± 2.333 years.

In terms of Body Mass Index (BMI), a significant difference was noted between the groups (p = 0.0084). The control group had a mean BMI of 26.94 ± 0.5472 kg/m², whereas the colon cancer patient group exhibited a higher mean BMI of 29.17 ± 0.6181 kg/m².

Measurement of TNF-α Levels

The serum levels of tumor necrosis factor-alpha (TNF-α) were quantified using the ELISA method. The findings of this study demonstrate a significant elevation in blood TNF-α levels (ng/L) in patients diagnosed with colon cancer in comparison to the control group (p < 0.001). Specifically, the mean TNF-α levels were 131.7 ± 8.546 ng/L in the control group and 215.2 ± 13.21 ng/L in the patient group, as illustrated in Figure 1.

Gender-Based Comparison of TNF-α Levels

The analysis revealed no significant difference in TNF-α levels between male patients (203.1 ± 20.57 ng/L) and female patients (222.4 ± 17.31 ng/L) with colon cancer. Similarly, the control group showed no substantial disparities in TNF-α levels among males (121.1 ± 13.75 ng/L) and females (139.6 ± 10.81 ng/L). However, significant differences were observed between male patients and both healthy genders, as well as between female patients and both healthy genders, as shown in Figure 2 and Table 2.

Comparison of TNF-α Levels Across Disease Stages and Treatment Modalities

The analysis of TNF-α levels among colon cancer patients revealed no statistically significant variation across different stages of the disease, indicating that disease progression does not appear to influence TNF-α levels.

Furthermore, when examining TNF-α levels based on treatment modalities, no substantial differences were observed among individuals receiving various therapies for colon cancer. The mean serum TNF-α levels for patients undergoing different treatments were as follows: 235.2 ± 31.96 ng/L for those receiving biological therapy

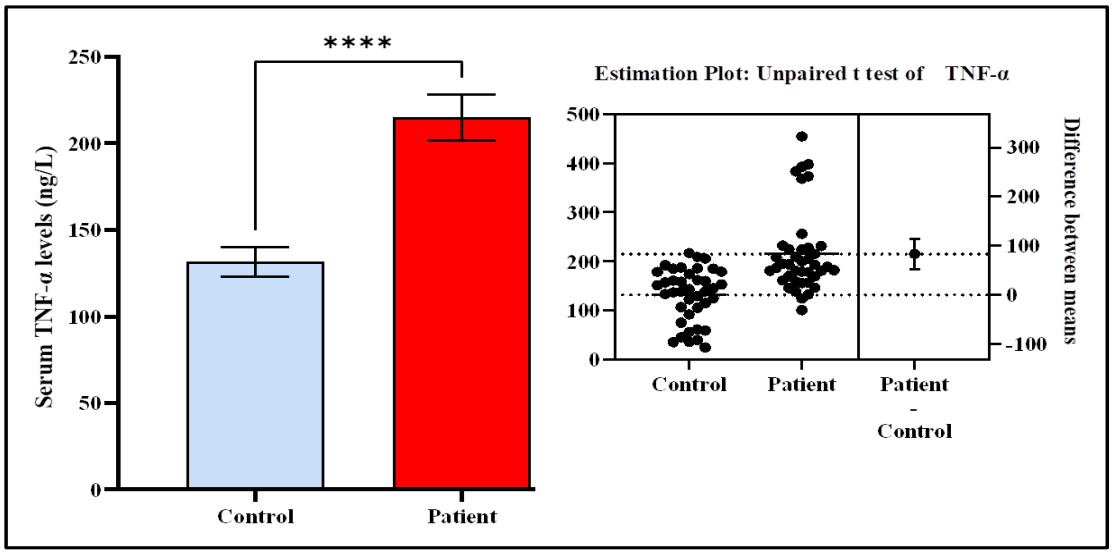

Figure 1. The Comparison of Mean Serum Tumor Necrosis Factor Alpha (TNF-α) Levels between the Control Group and Patients with Colon Cancer Reveals a Significant Difference. The figures illustrate a highly significant difference (p-value < 0.0001) in patients with colon cancer compared to the control group. Data are presented as means ± SEM. The asterisk (*) indicates a significant difference with P≤0.05.

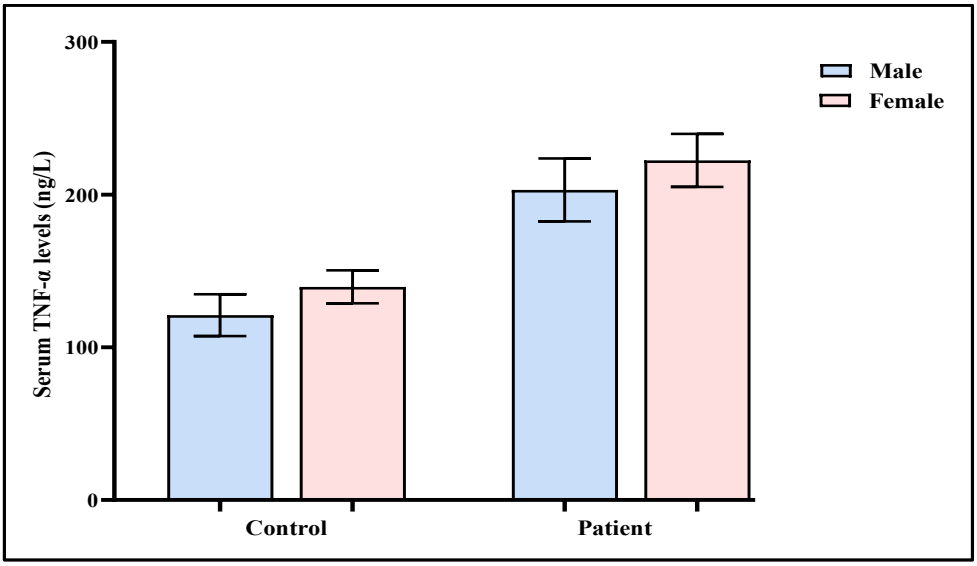

Figure 2. Comparison of Mean Serum TNF-α for Patients with Colon Cancer based on Differences between Males and Females

Table 2. Explains a Gender-based Comparison of the Average TNF-α Levels in Serum between Individuals with Colon Cancer and the Control Group.

Tukey's multiple comparisons test	Summary	Adjusted P Value
Control (Male vs. Female)	ns	0.8445
Control Male vs. Male patients	**	0.0085
Control Male vs. Female patients	***	0.0001
Control Female vs. Male patients	*	0.041
Control Female vs. Female patients	***	0.0007
Patient (Male vs. Female)	ns	0.8355

(n=10), 206.1 ± 16.78 ng/L for chemotherapy (n=13), 191.2 ± 45.19 ng/L for immunotherapy (n=6), and 220.8 ± 24.53 ng/L for surgical treatment (n=11). Statistical analysis confirmed these findings, showing no significant differences across treatment groups (P = 0.75140) (Table 3).

Overall, these results indicate that neither the stage of colon cancer nor the treatment modality significantly impacts serum TNF-α levels among the patients studied. Supplementary file contains additional information including Tables and Figures.

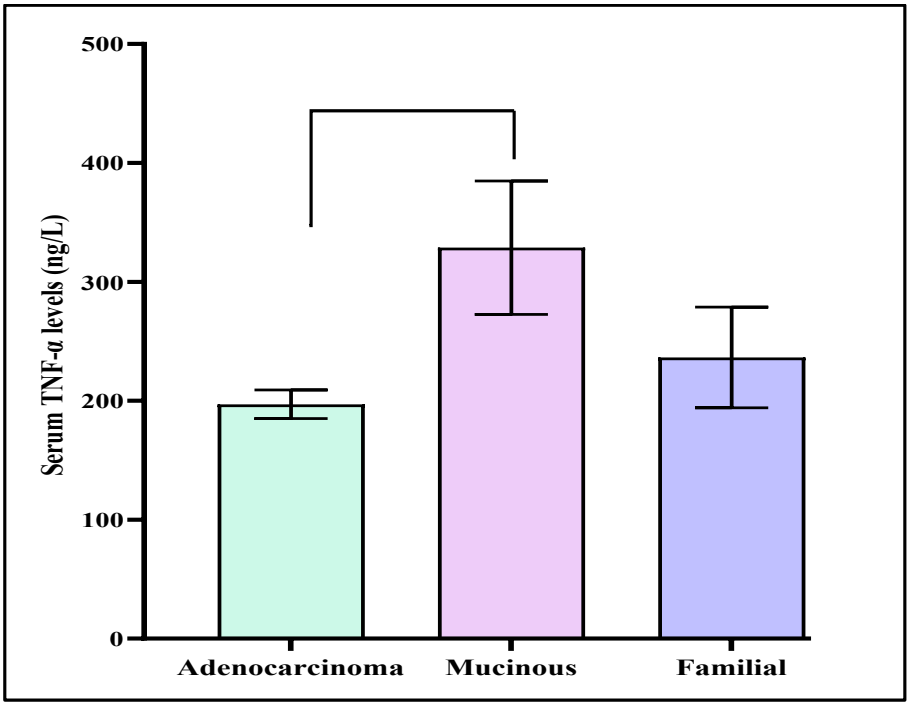

Figure 3. Comparison of TNF-α Concentration based on Colon Cancer Type. The figure illustrates significantly higher TNF-α levels in mucinous colon cancer compared to colon adenocarcinoma, with no significant differences observed between other groups.

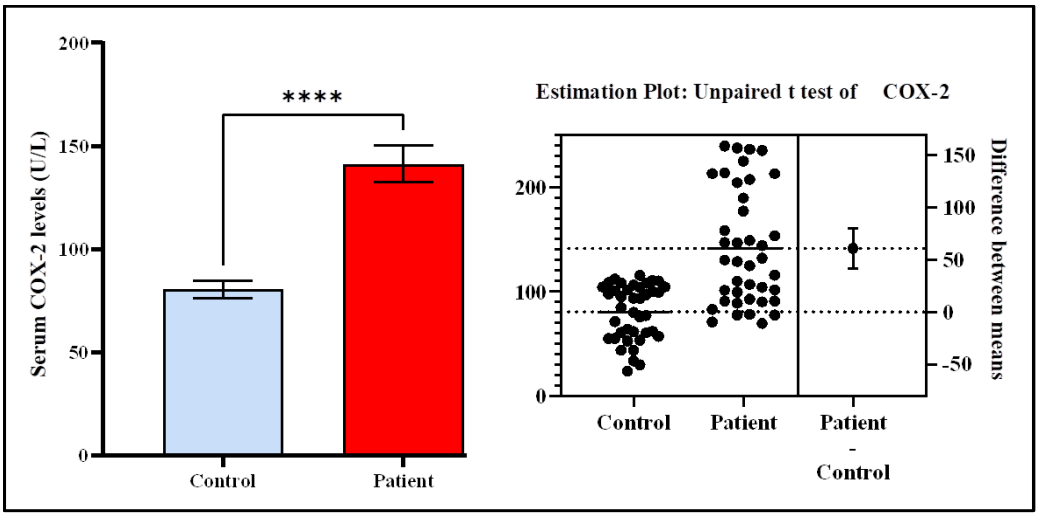

Figure 4. Comparison of Mean Serum COX-2 Levels between the Control Group and Colon Cancer Patients, Showing a Significant Difference. The figure displays a highly significant difference (p-value < 0.0001) in patients with colon cancer compared to the control group. Data are expressed as means ± SEM, indicating significance at $P \leq 0.05$.

Table 3. Explains a Gender-based Comparison of the Average COX-2 Levels in Serum between Individuals with Colon Cancer and the Control Group

Tukey's multiple comparisons test	Summary	Adjusted P Value
Control (Male vs. Female)	ns	0.998
Control Male vs. Male patients	***	0.0002
Control Male vs. Female patients	***	0.0004
Control Female vs. Male patients	****	<0.0001
Control Female vs. Female patients	***	0.0002
Patient (Male vs. Female)	ns	0.858

Table 4. Explains a Gender-based Comparison of the Average NOX1 Levels in Serum between Individuals with Colon Cancer and the Control Group

Tukey's multiple comparisons test	Summary	Adjusted P Value
Control Male vs. Female	ns	0.449
Control Male vs. Male patients	***	0.0006
Control Male vs. Female patients	***	0.0002
Control Female vs. Male patients	*	0.0236
Control Female vs. Female patients	*	0.0123
Patient Male vs. Female	ns	0.9979

TNF-α Levels Based on Cancer Type

A significant disparity in TNF-α levels was observed among patients based on the type of colon cancer. Specifically, the mean serum TNF-α levels were 197.1 ± 66.97 ng/L for patients with colon adenocarcinoma, 236.5 ± 94.65 ng/L for those with familial colon cancer, and 328.8 ± 112.1 ng/L for patients with mucinous colon cancer. Statistical analysis revealed that the difference between colon adenocarcinoma and mucinous colon cancer was statistically significant ($p = 0.0059$), indicating

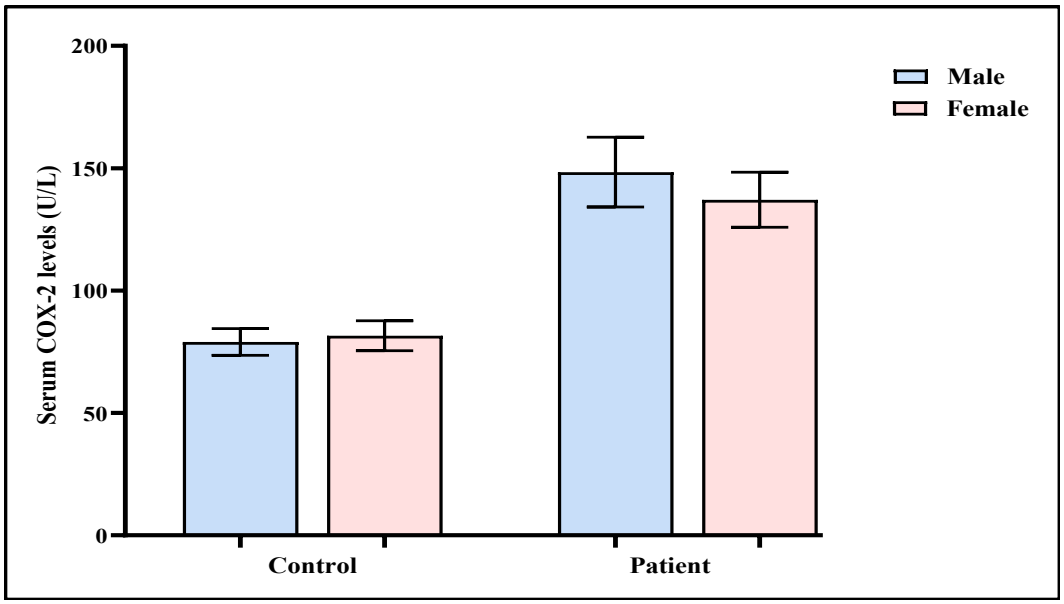

Figure 5. Comparison of Mean Serum COX-2 Levels in Colon Cancer Patients based on Gender Differences

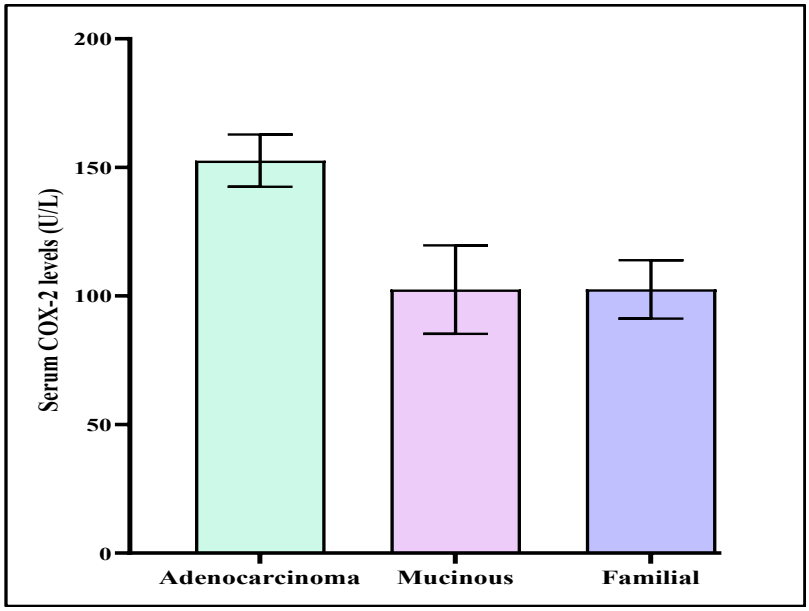

Figure 6. Mean COX-2 Levels Recorded for Different Types of Colon Cancer Show Significant Variation. In patients with colon adenocarcinoma (n=31), the mean COX-2 level was 152.6 ± 10.16, whereas in those with familial colon cancer (n=5), the mean was 102.5 ± 11.33. Similarly, patients with mucinous colon cancer (n=4) exhibited a mean COX-2 level of 102.5 ± 17.19. The observed ranges of COX-2 levels were as follows: 69.53 - 239.4 for colon adenocarcinoma, 78.2 - 144.1 for familial colon cancer, and 77.59 - 153.4 for mucinous colon cancer.

higher TNF-α levels in patients with mucinous colon cancer compared to those with colon adenocarcinoma. These findings suggest that TNF-α levels vary significantly depending on the type of colon cancer, particularly highlighting the elevated levels in patients with mucinous colon cancer Figure 3

Measurement of Serum COX-2

The study findings indicate that colon cancer patients exhibit significantly higher levels of cyclooxygenase-2 (COX-2) at (141.3 ± 8.759) U/L compared to the control group with (80.46 ± 4.175) U/L. Results demonstrate a statistically significant difference (p-value < 0.0001) in COX-2 concentrations between the control and cancer patient groups, as illustrated in Figure 4 .

Gender-Based Comparison of COX-2 Levels

No significant difference was observed in COX-2 levels between male patients (148.4 ± 14.24) U/L and female patients (137.1 ± 11.25) diagnosed with colon cancer. Additionally, there were no substantial disparities in COX-2 levels among controls, with males at (79.02 ± 5.49) U/L and females at (81.52 ± 6.116) U/L. However, statistically significant differences were noted between male patients and both genders in the control group, as well as between female patients and the controls, as represented in Figure 5 and Table 4.

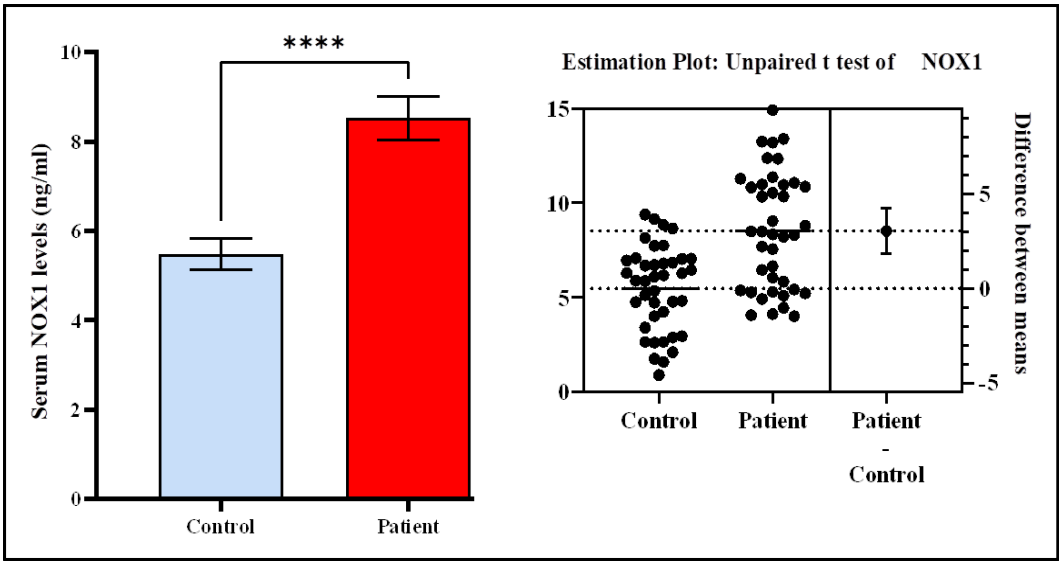

Figure 7. Mean NADPH oxidase 1 (NOX1) Levels Recorded for Colon Cancer Patients and Control Subjects. The figures show a highly significant difference (p-value < 0.0001). Data are expressed as means ± SEM.

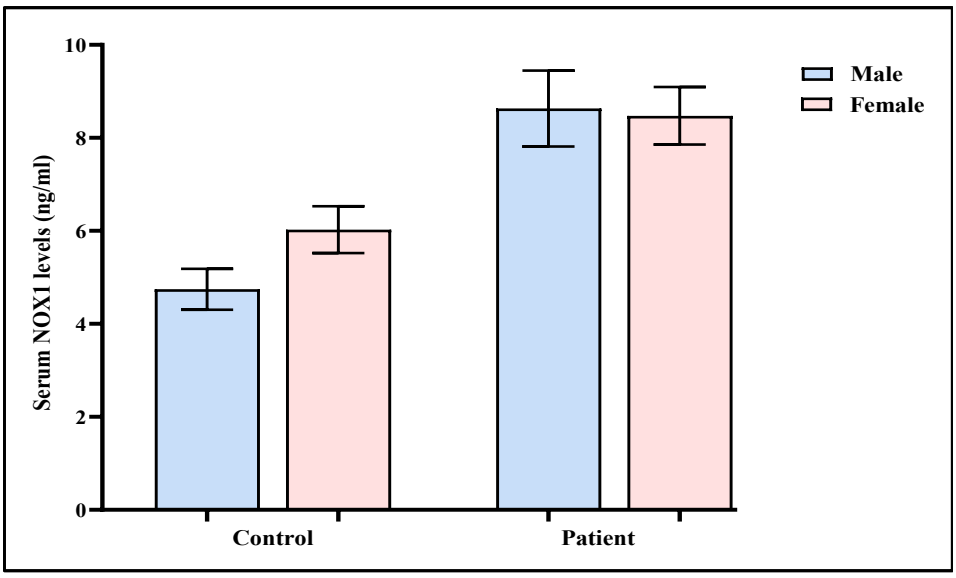

Figure 8. Comparison of Mean Serum NOX1 Levels in Colon Cancer Patients based on Gender Differences.

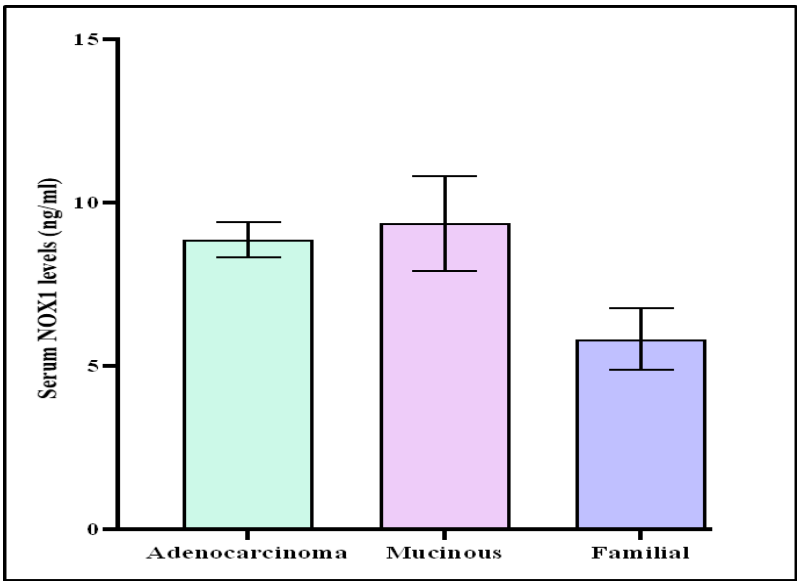

Figure 9. Comparison of NOX1 Concentrations among Different Types of Colon Cancer. The mean NOX1 levels recorded for various cancer types were as follows: Colon Adenocarcinoma (n=31) exhibited a mean level of 8.862 ± 0.5538, while Familial Colon Cancer (n=5) showed a mean level of 5.828 ± 0.9313, and Mucinous Colon Cancer (n=4) had a mean NOX1 level of 9.363 ± 1.443. The observed ranges of NOX1 levels were 4 - 14.92 for colon adenocarcinoma, 4.06 - 9.04 for familial colon cancer, and 5.84 - 12.34 for mucinous colon cancer.

COX-2 Levels

Comparison Across Disease Stages and Treatment Modalities

The evaluation of COX-2 levels (U/L) in colon cancer patients revealed no statistically significant variation across different disease stages, with an overall P value of 0.4048. Mean COX-2 levels were as follows: Stage 1 (n=11) 137.7 ± 15.55, Stage 2 (n=13) 153.2 ± 15.46, Stage 3 (n=11) 120.7 ± 16.5, and Stage 4 (n=5) 163.8 ± 28.85. The ranges for each stage were 69.53 - 237.3 (Stage 1), 78.2 - 236.1 (Stage 2), 77.59 - 239.4 (Stage 3), and 90.05 - 235 (Stage 4).

Regarding treatment modalities, COX-2 levels were also consistent across different therapies. The overall P value for treatment comparisons was 0.0843. Mean COX-2 levels were as follows: biological therapy (n=10) 108.9 ± 12.68, chemotherapy (n=13) 153.2 ± 15.46, immunotherapy (n=6) 176.4 ± 26.71, and surgical treatment (n=11) 137.7 ± 15.55. Observed ranges were 77.59 - 204.3 (biological therapy), 78.2 - 236.1 (chemotherapy), 90.05 - 239.4 (immunotherapy), and 69.53 - 237.3 (surgical treatment). These findings suggest that COX-2 levels do not significantly differ by disease stage or treatment modality (Figure 6). Supplementary file contains additional information including Tables and Figures .

COX-2 Levels Based on Cancer Type

A discrepancy in COX-2 levels was observed among patients with colon cancer based on the type of cancer.

Although the P value was recorded as 0.0537, indicating a trend toward significance, no statistically significant differences were found between the groups (ns). These findings suggest that COX-2 levels may vary depending on the cancer type, with colon adenocarcinoma showing higher levels compared to both familial and mucinous colon cancers (Figure 7).

Measurement of NOX1

The study's findings indicate that colon cancer patients had higher levels of NADPH oxidase 1 (NOX1) (8.533 ± 0.4872 ng/ml) than control subjects (5.481 ± 0.3551 U/L). The results indicated a significant difference (p-value < 0.0001) between the NOX1 concentrations in the control group and colon cancer patients (Figure 8).

Gender-Based Comparison of NOX1 Levels

No significant difference was observed in NOX1 levels between male patients (8.631 ± 0.8171 ng/ml) and female patients (8.474 ± 0.619 ng/ml) diagnosed with colon cancer. Additionally, there were no substantial disparities in NOX1 levels among controls, with males at (4.744 ± 0.4397 ng/ml) and females at (6.026 ± 0.503 ng/ml). However, statistically significant differences were noted between male patients and both genders in the control group, as well as between female patients and the controls, as represented in Figure 8 and Table 4.

NOX1 Levels: Comparison Across Disease Stages and Treatment Modalities

The evaluation of NOX1 levels (ng/L) in colon cancer patients revealed no statistically significant variation across different disease stages, with an overall P value of 0.9224. This indicates that NOX1 levels remain relatively consistent across the disease stages. The mean NOX1 levels recorded for each stage were as follows: Stage 1 (n=11) had a mean level of 8.401 ± 0.973, Stage 2 (n=13) displayed 8.852 ± 0.7963, Stage 3 (n=11) showed 8.077 ± 1.048, and Stage 4 (n=5) had a mean level of 8.998 ± 1.441. The ranges for each stage were 4.12 - 13.25 for Stage 1, 5.29 - 13.41 for Stage 2, 4 - 14.92 for Stage 3, and 4.45 - 13.21 for Stage 4 (Figure 9).

In addition, the analysis of NOX1 levels concerning treatment modalities indicated no substantial differences among individuals undergoing various therapeutic approaches for colon cancer, with an overall P value of 0.9223. The mean NOX1 levels for each treatment group were as follows: Biological Therapy (n=10) had a mean level of 8.034 ± 1.158, Chemotherapy (n=13) displayed 8.852 ± 0.7963, Immunotherapy (n=6) showed 8.917 ± 1.18, and Surgical Treatment (n=11) had a mean level of 8.401 ± 0.973. The observed ranges of NOX1 levels were 4 - 14.92 for Biological Therapy, 5.29 - 13.41 for Chemotherapy, 4.45 - 13.21 for Immunotherapy, and 4.12 - 13.25 for Surgical Treatment.Overall, these findings indicate that NOX1 levels do not significantly vary by disease stage or treatment modality in colon cancer patients. Supplementary file contains additional information including Tables and Figures.

NOX1 Levels Based on Cancer Type

The analysis of NOX1 levels highlighted a lack of statistically significant variation among patients with different types of colon cancer. The P value for these comparisons was 0.1037, indicating no significant differences between the cancer types (ns).

Discussion

Colon cancer is a multifactorial disease driven by genetic, epigenetic, and environmental factors, with chronic inflammation playing a pivotal role in its initiation and progression. Among the key mediators of inflammation and oxidative stress, COX-2, NOX1, and TNF-α have emerged as critical players in the tumor microenvironment. Their dysregulation contributes to tumorigenesis through distinct but interconnected pathways, including the promotion of cell proliferation, survival, angiogenesis, and immune evasion. COX-2, an inducible enzyme responsible for the conversion of arachidonic acid to prostaglandins, is overexpressed in the majority of colorectal cancers [16, 3]. Its upregulation is associated with increased levels of prostaglandin E2 (PGE2), a key mediator of inflammation and tumorigenesis. PGE2 promotes colon cancer progression by enhancing cell proliferation, inhibiting apoptosis, and stimulating angiogenesis. Furthermore, COX-2-derived PGE2 has been shown to modulate the tumor microenvironment by recruiting immunosuppressive cells, such as myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs), thereby facilitating immune evasion. The overexpression of COX-2 in colon cancer is often driven by inflammatory cytokines, including TNF-α, and reactive oxygen species (ROS) generated by NOX1. This highlights the interplay between COX-2 and other inflammatory mediators in promoting tumorigenesis [17, 18]. Importantly, COX-2 inhibitors, such as nonsteroidal anti-inflammatory drugs (NSAIDs), have demonstrated chemopreventive effects in CRC, underscoring the therapeutic potential of targeting this pathway. However, the cardiovascular risks associated with long-term COX-2 inhibition necessitate the development of safer and more selective therapeutic strategies. NOX1, a member of the NADPH oxidase family, is a major source of ROS in colon epithelial cells. While physiological levels of ROS are essential for cellular signaling, excessive ROS production by NOX1 contributes to oxidative stress, DNA damage, and genomic instability, all of which are hallmarks of cancer. In colon cancer, NOX1 is frequently upregulated and has been implicated in promoting cell proliferation, migration, and invasion through the activation of redox-sensitive signaling pathways, such as MAPK, NF-κB, and PI3K/Akt [19, 20]. The crosstalk between NOX1 and COX-2 further amplifies the pro-tumorigenic effects of oxidative stress and inflammation. For instance, ROS generated by NOX1 can activate *COX-2* expression, while COX-2-derived PGE2 can enhance NOX1 activity, creating a positive feedback loop that sustains chronic inflammation and tumor progression. Targeting NOX1, either alone or in combination with COX-2 inhibitors, represents a promising therapeutic approach to disrupt

this vicious cycle and mitigate oxidative stress in colon cancer. TNF-α, a pro-inflammatory cytokine produced by immune cells and tumor-associated stromal cells, plays a dual role in colon cancer. On one hand, TNF-α can induce apoptosis in cancer cells through the activation of death receptors and caspase signaling. On the other hand, chronic exposure to TNF-α promotes tumorigenesis by activating NF-κB and STAT3 signaling pathways, which drive inflammation, cell survival, and proliferation [21, 22]. Elevated levels of TNF-α in the tumor microenvironment are associated with increased COX-2 expression and NOX1 activity, further linking inflammation and oxidative stress to colon cancer progression. In addition to its direct effects on cancer cells, TNF-α modulates the tumor microenvironment by promoting angiogenesis, recruiting inflammatory cells, and enhancing the expression of adhesion molecules that facilitate metastasis. Anti-TNF-α therapies, such as monoclonal antibodies, have shown efficacy in inflammatory bowel disease [23], a condition that predisposes to colon cancer. However, their role in CRC treatment remains controversial, as TNF-α inhibition may impair anti-tumor immunity in certain contexts. The interplay between COX-2, NOX1, and TNF-α underscores the complex network of inflammation and oxidative stress in colon cancer [24, 25]. TNF-α-induced activation of NF-κB and AP-1 transcription factors upregulates COX-2 and NOX1 expression, while ROS generated by NOX1 further amplifies TNF-α signaling. This synergistic interaction creates a pro-tumorigenic microenvironment characterized by sustained inflammation, oxidative stress, and immune suppression. Therapeutically, targeting these pathways in combination may offer synergistic benefits. For example, dual inhibition of COX-2 and NOX1 could simultaneously reduce prostaglandin-mediated inflammation and ROS-induced DNA damage, while anti-TNF-α therapy could attenuate the upstream inflammatory signals driving their expression [26, 27]. However, the potential for adverse effects, such as impaired wound healing and immune suppression, must be carefully considered in the design of combination therapies. COX-2 and Its Role in Colon Cancer. The results of our study demonstrated elevated levels of COX-2 in colon cancer patients compared to healthy controls. This increase can be attributed to the activity of deoxycholic acid in colorectal cancer cells, which stimulates COX-2 expression. COX-2, in turn, promotes the production of prostaglandins from arachidonic acid, contributing to tumor formation. These findings align with previous research showing that COX-2, a human cyclooxygenase, plays a key role in inflammation and tumorigenesis by converting arachidonic acid into eicosanoids, including prostaglandins, prostacyclins, and thromboxanes [28, 29]. Elevated COX-2 expression has been observed in several cancers, including lung, breast, stomach, colon, prostate, and pancreas cancers [28]. In colon cancer, COX-2 expression is significantly higher in tumor tissues compared to adjacent normal mucosa, correlating with increased prostaglandin production. This increase is often stimulated by mitogens or tumor promoters [29]. Animal studies have further supported these findings, showing that intestinal tumors induced by carcinogens exhibit elevated COX-2 protein and mRNA levels, while normal mucosa in these models lacks COX-2 expression [30]. Interestingly, studies have demonstrated that the inhibition of COX-2 can reduce tumor incidence and burden. For instance, the administration of celecoxib, a specific COX-2 inhibitor, significantly decreased the frequency and incidence of azoxymethane (AOM)-induced colon tumors in mice, with tumor burden reduced by over 87% [31, 32]. These results suggest that COX-2 not only plays a role in tumorigenesis but also represents a potential therapeutic target for colon cancer. An elevated percentage of NADPH oxidase 1 (NOX1) was found in colon cancer patients, consistent with previous studies. Overexpression of NOX1 leads to increased reactive oxygen species (ROS) generation, which contributes to cellular transformation and tumor progression. ROS produced by NOX1 not only facilitates the growth and survival of cancer cells but also enhances the production of proinflammatory cytokines [33]. Studies have shown that NOX1 overexpression in colon cancer is linked to oxidative stress and increased proliferation of cancer stem cells. NOX1-mediated ROS production has been identified as a key factor in activating signaling pathways, such as mTORC1, which promote tumorigenesis [34, 35]. Additionally, NOX1 plays a role in regulating NADPH levels, critical for cancer cell growth and survival through mechanisms involving AKT phosphorylation, p53 stimulation, and calmodulin interactions[36, 37]. Several members of the NOX family, including NOX1 and NOX4, are overexpressed in various cancers, particularly colorectal cancer [35]. While NOX1 promotes tumorigenesis, studies have also shown that the inhibition of NOX1 can prevent cancer cell proliferation and reduce ROS levels, highlighting its potential as a therapeutic target [38]. Our study confirmed significantly elevated levels of TNF-α in colon cancer patients compared to healthy controls. TNF-α, a key inflammatory cytokine, is known to play a pivotal role in the tumor microenvironment by promoting inflammation, angiogenesis, and tumor proliferation [39]. In colon cancer, TNF-α contributes to cancer cell migration and invasion through activation of the ERK signaling pathway and regulation of proteins such as TROP-2 [40, 41]. Previous studies have shown that TNF-α levels are elevated in inflammatory conditions, such as Crohn's disease and ulcerative colitis, which are risk factors for colon cancer [42]. TNF-α-induced inflammation also stimulates COX-2 expression, further driving tumor formation [43]. Interestingly, TNF-α is often expressed by cancer cells as a mechanism for immune escape, inducing programmed cell death in immune cells and reducing immune response to the tumor [44]. Elevated plasma cytokine levels, including TNF-α, have been shown to predict poor clinical outcomes in patients with advanced colon cancer [45]. Despite its well-known inflammatory properties, TNF-α has also been shown to enhance the migration and invasion of colon cancer cells, making it a critical factor in cancer progression [32, 33]. The association between TNF-α and TROP-2 expression further underscores its role in promoting tumor spread and metastasis [40, 41].

In conclusion, our findings confirm that COX-2, NOX1, and TNF-α are significantly elevated in colon cancer patients and play critical roles in inflammation, oxidative stress, and tumor progression. These biomarkers not only provide insights into the molecular mechanisms underlying colon cancer but also represent potential targets for early detection and therapeutic intervention. Future studies should further explore the pathways involving these molecules to develop more effective strategies for colon cancer management.

Limitations of the study

There were a number of problems faced in conducting this research, including the difficulty of obtaining samples from patients to conduct the research and the lack of previous research studies on this subject. In addition, the size of the sample collected was small and insufficient, the high financial cost, and the short time constraints.

Author Contribution Statement

Ashwaq Nouri Mawat and Dr. Zainab Al-Abadi participated in the design of the study. Analyze the results and draft the report.The authors gave their approval . The final version to be submitted

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The authers ininvoleed in the research

Ashwaq Nouri Mowat and Dr. Zainab Al-Abadi contributed to the research design. To analyze the results and write the manuscript. The authors approved the final version for submission

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Conflict of interest statement

The authors have no conflict of interest with respect to the publication of this article.

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