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International Journal of Medical Science and Dental Health (ISSN: 2454-4191)
Volume 11, Issue 11, November 2025
Doi: https://doi.org/10.55640/ijmsdh-11-11-07

Correlation Between Serum Levels of Tumor Necrosis Factor-Alpha (TNF-A) And Histopathological Grading of Colorectal Cancer

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Received: 16 October 2025, **accepted:** 21 October 2025, **Published Date:** 10 November 2025

Abstract

Background: Colorectal cancer is one of the most common malignancies in the world and the fourth leading cause of cancer death. Inflammation of long-term duration continues to be the disease's initiating and progressing factor, as tumor necrosis factor-alpha and several cytokines are regulators of its growth. This factor has been shown to stimulate the growth of sarcoma cells, angiogenes growth, and metastasizes the ROS-producing system to stimulate sarcoma cells. The connections among the growth of TNF-α and histopathological grading in colorectal tumors were not calculated. The purpose of the study: to assess the serum level of TNF-α in patients with colorectal sarcoma compared to a control group and to determine the relationship between TNF-α amounts in the tissues and histopathological benignant sarcoma grading.

Materials and methods: We have conducted a case–control survey in Middle Euphrates Oncology Center, Najaf, Iraq, from December 2023 through April 2024. This study enrolled 62 individuals whose first diagnosis was histopathological verification of colorectal sarcoma and 48 age- and sex-matched healthy people. Furthermore, people with chronic systemic diseases, autoimmune illnesses, or other malignant tumors were removed from the test. Venous blood samples have been gathered before the launch of any chemotherapy or radiotherapy. A human ELISA kit has examined the TNF-α content in the serum, according to the manufacturer's instruction.

Results: Mean serum TNF-α levels were significantly higher in CRC patients (42.6 ± 11.3 pg/mL) than in controls (35.8 ± 9.7 pg/mL) ($p = 0.034$). Furthermore, TNF-α concentrations demonstrated a significant stepwise increase with tumor dedifferentiation: Grade I (37.8 ± 8.4 pg/mL), Grade II (43.9 ± 10.2 pg/mL), and Grade III (49.6 ± 11.1 pg/mL), with the intergroup difference reaching statistical significance ($p = 0.022$). These findings indicate a positive correlation between TNF-α expression and histopathological severity in colorectal cancer.

Conclusion: Elevated levels of TNF-α have been implicated with the presence and histological advancement of CRC. With rising tumor dedifferentiation, TNF-α broadens, which could be associated with a rise in inflammatory and oncogenic signaling in the worst CRC phenotypes. Hence, TNF-α stands as a potential biomarker of tumor grade and therapeutic monitoring, highlighting its role in the pathogenesis of CRC.

Keywords: TNF-α, periodontitis, Colorectal Cancer, Histopathological Grades

Introduction

Furthermore, it is true that chronic inflammation is one of the most overlooked hallmarks of cancer; however, it fills the gap between the known connections among chronic immune activation, oxidative stress, and tissue regeneration, allowing neoplastic cell development and metastasis. To put this

another way, in this way, inflammatory cytokines, reactive oxygen species, and growth factors can target one another, causing damage to DNA, proliferative signals equivalent to those caused by mitogenesis, inadequate apoptotic control, and finally creating a tumor-useful milieu. The GI tract is more severely impacted; for example, chronic colon inflamed illness patients are believed to have a vastly heightened risk of colon cancer, and research data suggests that chronic inflammation can contribute to colon cancer formation (Bardelčíková et al., 2023).

Among a plethora of inflammatory mediators, Tumor Necrosis Factor- alpha occupies a unique dualistic position: TNF- α was first discovered and prioritized as a tumour necrosis agent; today, however, it is well-established that under some conditions, TNF- α may promote cancer. Major factors that testify for this ability are the initiation of NF-kappa B and STAT3 signaling through the chronic exposure to tumor necrosis factor. Moreover, it also enhances angiogenesis by inducing the epithelial to mesenchymal transition and affects immunological aspects of the TME by influencing immune cell recruitment. The duality of this factor, beneficial at high doses, and venomous under low acute doses, ensures that the TNF- α factor is of vital interest to researchers (Montfort et al., 2019).

In the case of CRC specifically, abundant evidence indicates a TNF-α expression in neoplastic tissues and the serum of afflicted patients. Zheng et al. reported a study of 162 CRC patients and 105 healthy subjects, conducted to ascertain the correlation of TNF-α levels with different clinical factors. The authors found that CRC cases had serum TNF-α levels significantly higher compared to healthy subjects and that those high TNF-α values correlated with poor differentiation. Still, such observational evidence, even adjusted to account for stage in multiscale quantitative models, does not prove the causative effect. In this study, utilizing the co-expression network scores for the sphere network genes and a tissue sample, and matching the TNF-α levels, showed that while there is a chance for the TNF-α to be a bystander biomarker or simply be secondary to progression, there is also a significant chance that the circulating TNF-α levels could serve as a systemic biomarker for cancer susceptibility (Zheng et al., 2022).

Histopathological grading is a fundamental component of the prognostic assessment While TCGA grading is a crucial component of the prognostic workup in CRC – reflecting the degree of differentiation of a tumour, cellular atypia, and the native biological invasiveness of a tumour, and therefore the outcome. However, the TNM staging, while reflecting the spread, does not truly correlate with histology and gives no information on the biology and maturation of a tumour (Barresi et al., 2015). As such, the question of whether serum TNF-α correlates with grade is relevant to clinical practice – if raised TNF-α actually reflects worse differentiation, this could assist stratifying pre-op patients, improve our models, perhaps allow us to investigate the mechanisms underlying the inflammation-driven process of tumour dedifferentiation. However, while some studies have investigated the matter between TNF-α measurements in CRC and stage, more histologic proceeding, actual few have included true actual histology as the endpoint, and the results, as seen, contradict (Florescu et al., 2023).

Bridging this gap would be important both from mechanistic and translational viewpoints. Mechanistically, a graded dependence of TNF-α release on tumour degree would support the model that chronic inflammatory signalling does not just boost tumour growth but renders tumour progression more by enabling dedifferentiation. In translation, if serum TNF-α simply reflects histological degree, it may be utilized clinically as a “minimally invasive” adjunct biomarker to aid pre-operative decisions about the necessity of possible risk stratification, forgoing surgery, or involving adjuvant therapies. Due to the TNF-α complexities and the disparities among reports in terms of assays, personnel, and evaluation measures, a focused correlation investigation between TNF-α and histological grading in colorectal cancer is both timely and necessary. Consequently, the study is planned to observe the relationship between A- level and histopathological grading (Szylberg et al., 2019).

The present study aims to investigate the correlation between serum TNF-α levels and histopathological grading of colorectal cancer. By quantifying pre-operative TNF-α and correlating with blinded pathologic grade assessments, and adjusting for confounding factors such as stage and patient demographics.

Patients and Methods

A hospital-based case–control study was conducted at the Middle Euphrates Oncology Center, Al-Najaf, Iraq, between December 2023 and April 2024. The study assessed any association between serum levels of tumor necrosis factor-alpha and histopathological grading of CRC. A total of 110 participants were recruited, including 62 patients with a histological biopsy report of colorectal adenocarcinoma, also referred to as patients, and 48 apparently healthy individuals as controls. The diagnosis of CRC was made through clinical assessment and colonoscopy and confirmed with biopsies sent to the Histopathology Department, where certified

pathologists performed microscopic examination to assess the tumor type, differentiation, and grade. Participants aged 30–75 years were eligible for the study. The case group included patients who were diagnosed recently and had not received chemotherapy or radiotherapy. The control group was composed of healthy volunteers in the same age group and matching sex as the cases, who had no clinical or laboratory manifestation of any malignant or inflammatory disease. Exclusion criteria included chronic systemic diseases, including diabetes mellitus and cardiovascular, renal, and hepatic diseases, autoimmune disorders, inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, other simultaneous malignancies, and anti-inflammatory, immunosuppressive therapy, or biological therapy within three months before sampling extraction. SVrij participant was made aware of the study's purpose and procedures, and informed consent was obtained from each participant before initiation of the study.

Histopathological grading and sample collection

Prior to treatment, tumor tissue samples obtained from diagnostic biopsies or surgical resections were processed and examined by two independent pathologists blinded to the origin of the tissue. A histopathological grading was conducted been according to the World Health Organization, 2019 classification of colorectal adenocarcinoma, which is based on the percentage of glandular differentiation as follows:

- Grade I (Well differentiated) – >95% gland formation
- Grade II (Moderately differentiated) – 50–95% gland formation
- Grade III (Poorly differentiated) – <50% gland formation

In cases of metastases, histological features were not considered. If two pathologists assessed the tumor differently, they reviewed the sections together to achieve consensus. Venous blood samples out of 5 mLs were gathered from both patients and healthy controls by a sterile plain tube between 8:00 -10:00 a.m. fasting overnight to reduce variation and diurnal variations. The samples were allowed to clot at room temperature for a duration of 30 minutes and subsequently centrifuged at 3000 rpm for 10 minutes to separate serum. Serum was aliquoted to Eppendorf sterile tubes then stored at −80°C until analysis was completed.

Estimation of Serum TNF-α Levels

Serum TNF-α concentrations were determined by a quantitative sandwich enzyme-linked immunosorbent assay kit according to the manufacturer's instructions. Analytical accuracy and reproducibility were confirmed by repeat standardized measurement of serial dilutions and controls in duplicate. The optical density at 450 nm was recorded using a microplate reader and expressed as a concentration based on a standard curve.

Ethical Considerations

The study was accepted by the Ethical Committee of Middle Euphrates Oncology Center, Al-Najaf, Iraq (Approval No.: 122. In 12\1\2025. And, Also, the study was done complying with the declaration of Helsinki 2013. The participants were familiarized with the goal of the study and the procedure and the risk as well as benfeits and all of them agreed to participate and their privacy were secured.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). The association between TNF-α concentration and histopathological grade was assessed using one-way ANOVA (Al-fahham, 2018). Correlation strength was evaluated using Spearman's correlation coefficient (ρ). A p-value < 0.05 was considered statistically significant.

Results

The age and gender distribution of the patients and controls is summarized in Table 1. As demonstrated above, the resistant groups are generally well-balanced with the target population with respect to the age, sex, smoking, and residence. It can be identified from Table 1 that the most common age group in the two samples was 51-64 years. Such an age composition when patients correlate positively with the incidence of colorectal cancer, with its risk increasing with age. The comparison of age distribution was not statistically different (X 2 = 0.28, > 0.05) but all age-matched. The sex distribution also showed a small overrepresentation of men in the population of both groups 59.7% and 52.1%, respectively. At the same time is a well-known fact that colorectal cancer mainly affects men in 55 cases. This variation was also non-statistically different X 2 = 0.16, > 0.05. Smoking was also not a confounder factor in this sample since there were more than 30% of CRC patients and 25% of the control smoking population. Although people widely know that smoking affects the development of colorectal cancer, this distribution was non-statistically different X 2 = 0.33, > 0.05. In the analyzed target population, the urban and rural distribution was also not different 3 > 0.05. Two-thirds of the CRC patients and the control group were occupied in the town.

Table 1. Distribution of general information for both CRC patients and control

Indicators		`Patients (No. = 62)		Control (No. = 48)		Chi Square	P value (Sig.)
		Freq.	%	Freq.	%		
Age/Years	44-50	12	19.4	11	22.9	0.28	>0.05 (NS)
	51-57	16	25.8	13	27.1		
	58-64	18	29	12	25		
	≥ 65	16	25.8	12	25		
Gender	Male	37	59.7	25	52.1	0.16	>0.05 (NS)
	Female	25	40.3	23	47.9		
Smoking	Yes	21	33.9	13	27.1	0.33	>0.05 (NS)
	No	41	66.1	35	72.9		
Residence	Urban	40	64.5	31	64.6	0.33	>0.05 (NS)
	Rural	22	35.5	17	35.4		

The CRC patient cohort in the present study was histopathologically classified into groups by the grade of differentiation, and the patients' distribution was quantified. As illustrated in Figure 1, the largest subgroup was represented by Grade II differentiation, corresponding to 32 patients and 51.6%, which indicated a prevalence of cases with an intermediate degree of glandular formation and cellular atypia. Grade I differentiation with 29.0% occurred in 18 patients and indicated lesions with relatively preserved glandular architecture and mild nuclear pleomorphism. In turn, the smallest subgroup was constituted by Grade III differentiation, which occurred in 12 patients with 19.4%. This subgroup was characterized by a high degree of cellular anaplasia and minimal glandular formation.

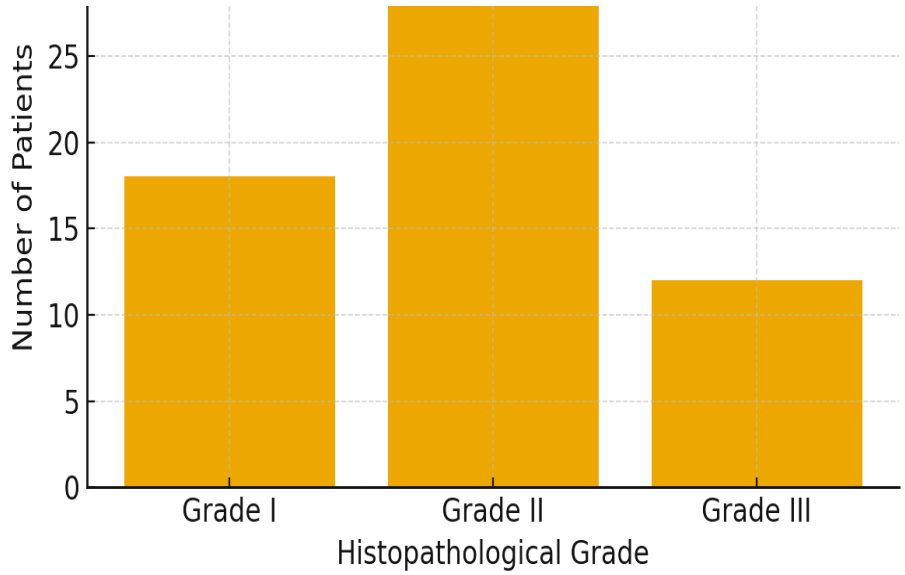

Figure 1. The distribution of colorectal cancer patients (n = 62) according to histopathological grades

The mean serum TNF-α concentration in colorectal cancer patients (42.6 ± 11.3 pg/mL) was notably higher than that observed in healthy controls (35.8 ± 9.7 pg/mL). Statistical analysis using an independent-samples t-test revealed a

significant difference between the two groups (p = 0.034), indicating an elevated systemic inflammatory state among CRC patients (table 2).

Table 2. Comparison of TNF-α levels between CRC patients and control participants

Groups	No.	TNF-α (pg/ml) Mean ± SD	T Test (P Value)
Patient	62	42.6 ± 11.3	(0.034)
Control	48	35.8 ± 9.7	

The mean serum TNF-α levels increased progressively with the histopathological grade of colorectal cancer. It was 37.8 ± 8.4 pg/mL in Grade I, 43.9 ± 10.2 pg/mL in Grade II, and 49.6 ± 11.1 pg/mL in Grade III. One-way ANOVA showed a statistically significant difference between these subgroups ascribed to the dissimilarity in the variation between the subgroups 16.5 3.8, p = 0.022. Therefore, the levels of TNF-α also increase with the grade of the tumor (table 3; figure 2).

Table 3. Differences in TNF-α among patients' subgroups classified according to histopathological grades of colorectal cancer

Subgroups		No.	TNF-α (pg/ml) Mean ± SD	F Test	P value (Sig.)
Grades	**Grade I**	**18**	**37.8 ± 8.4**	**16.5**	**0.022 (S)**
	Grade II	**32**	**43.9 ± 10.2**		
	Grade III	**12**	**49.6 ± 11.1**		

Discussion

In this case–control study, serum tumor necrosis factor-alpha (TNF-α) concentrations were significantly higher in colorectal cancer (CRC) patients (mean 42.6 ± 11.3 pg/mL) than in healthy controls (mean 35.8 ± 9.7 pg/mL; p = 0.034). In addition, TNF-α levels exhibited a graded increase across histopathological categories, with Grade III tumors having the highest circulating TNF-α, followed by Grade II and Grade I. These subgroup difference statistical analysis was significant. Therefore, our results indicate that systemic TNF-α is increased in CRC and correlates with poorer tumor differentiation, supporting the association between inflammation and more cancer phenotypes.

These findings confirm the notion that systemic inflammatory activation, as demonstrated by TNF-α, represents a common characteristic of colorectal cancer. The global enhancement of TNF-α in the patients when equated to the controls corresponds to various lately published clinical findings demonstrating higher circulating pro-inflammatory cytokines in the CRC entities. Florescu and others revealed higher TNF-α levels in addition to IL-1β and IL-6 among CRC sufferers and established interactions between inflammatory factors and multiple clinicopathological qualities, they also discovered that pre-therapy cytokine compositions differed between CRC sufferers and healthy persons and highlighted TNF-α's and cytokine's potential role as a marker panel (Florescu et al., 2023). Zheng et al., 2022).

From a mechanistic standpoint, TNF-α is poised to connect chronic inflammation with tumorigenesis. Recent reviews and experimental databases reported TNF-α capacity to induce NF-κB and STAT3 signaling, activate epithelial–mesenchymal transition and angiogenesis, and reprogram the tumor ecosystem, including immune components, which contribute to invasion, metastasis, and therapeutic resistance. TNF-α exposure leads to increased oncogenic miRNA- 21 and its targets, driving migration and inhibiting epithelial marker

expression in colorectal cancer cell lines in vitro, providing clear biological support to the clinical finding in our study of increased TNF-α equating with the more aggressive histology (Bahrami et al., 2024). Overall, these molecular data offer potential biological explanations for the graded rise in TNF-α levels with lesser degrees of differentiation observed here (Alotaibi et al., 2023; Montfort et al., 2023).

Concordance and heterogeneity when comparing the findings with previous clinicopathological studies are observed. Various observational works describe positive correlation between circulating TNF-α and TNF signaling with advanced stage or negative outcomes in CRC. For instance, Natkaniec et al. suggested correlations between TNF-α promoter variants and tumor stage/grade, meaning that higher TNF pathway activity might correlate with poor differentiation and relapse (Natkaniec et al., 2018; Khan et al., 2025). Our controlled recruitment of treatment-naïve cases and a systematic histopathological grading of the cases may have eliminated some of the sources of heterogeneity. This can partially explain why a clear graded relationship emerged in this body of work. In contrast, other series have reported weaker or non-monotonic associations between serum TNF-α and individual histological grades; this probably reflects between-study variations in assay platform sensitivity, patient ascertainment efforts, timing of the samples , and coexistent inflammatory comorbidity (Florescu et al., 2023; Zheng et al., 2022).

There are two essential clinical implications of this relation of higher TNF-α and poorer differentiation. Firstly, TNF-α (particularly TNF-driven transcriptional signatures) may be a minimally invasive addition to the lap of the preoperative care team when combined with the imaging and validated tumor markers. Suspiciously raised TNF-α TME levels could trigger a more in-depth diagnostic or therapeutic workup, theoretically. Given the direct mechanistic contribution of TNF-α to pro-tumorigenic signaling, the observations argue for further trials of anti-inflammatory or TNF-targeted interventions—alone or in combination with other therapies—in distinct populations of patients with the highly inflamed neoplasms. A therapeutic qualification based on an agnostic 'inflammation index' is indeed a feasible method. But since TNF-α and its effect on tumorigenesis exhibit a paradoxical behavior — it can either pat on the back or destroy the tumor, depending on the context — such a trial would be impossible without a hard selection of patients and a reliable set of mechanistic biomarkers (Montfort et al., 2019; Alotaibi et al., 2023).

Several study limitations warrant explicit consideration. To begin with, the cross-sectional design precludes any causal inference: a higher TNF-α might indicate the tumor biology that incites from inflammation, or it may be the cause of the dedifferentiation; longitudinal data would be essential to disentangle this directionality. Furthermore, even though the study preceded women with major chronic or autoimmune pathologies, there is always a possibility for residual confounding from a subclinical inflammatory condition or medication use. Similarly, the variability on cytokine measurements via assay sensitivity or pre-analytical handling can produce up to 25% variation in absolute values. We managed this by assaying all samples in duplicate under uniform circumstances, but this complicates comparisons between studies. Finally, concerning the observed differences, the sample size was sufficient, but expanding this in multi-center cohorts would validate this generalizability across populations, although a larger sample size would be needed.

Conclusion

The current study confirms that comparison to healthy controls CRC patients have high serum TNF-α while worsening histopathologic grade was encountered with a trend of increased TNF-α. The current study result in line with the multiple clinical and experimental evidences reporting that TNF-α is one of the contributors to the colorectal tumor progression in this concern TNF-α might be considered as prognostic or mechanistic alongside other biomarkers.

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