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The Role of Tumor Necrosis Factor-Alpha and C-C Motif Chemokine Ligand 3 Serum Levels in Some Iraqi Colorectal Cancer Patients

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Abstract

Background The second leading cause of cancer-related death globally, colorectal cancer (CRC) is the malignant neoplasm with the third highest diagnosis rate. It is the primary cause of cancer-related morbidity and mortality in Iraq and ranks second in terms of frequency of death after cardiovascular disorders. An inflammatory cytokine called tumor necrosis factor-alpha (TNF-α) plays a critical role in the immune response and may contribute to CRC development. Patients with CRC who had elevated serum levels of C-C motif chemokine ligand 3 (CCL3) had a worse prognosis than those who had low levels of this chemokine.

Objective To evaluate the role of TNF-α and CCL3 in CRC patients by assessing the serum level estimation of TNF-α and CCL3 in addition to the demographic data analysis.

Methods The study has been approved by the College of Medicine, Al-Iraqia University and included 100 samples from male and female patients from the Medical City, Al-Imamein Al-Kadhimein Medical City, Al-Amal Hospital, and also from Al-Bilad Private Hospital suffering from problems in the colon and rectum. The study lasted for five months (from November 2023 until March 2024). The participants were divided into 2 groups: the 1st one contained CRC patients, while the 2nd contained colorectal problems other than CRC. Five ml of blood was taken from each participant for estimation of TNF-α and CCL3 serum level by using enzyme-linked immunosorbent assay. Demographic data were collected from the study participants (such as age, gender, address, and medical history). Ten samples were discarded due to hemolysis.

Results The serum levels of TNF-α and CCL3 in CRC patients showed a higher significant difference ($P < 0.05$) compared with the 2nd group.

Conclusion TNF-α and CCL3 significantly appeared as indicators for the progression of CRC cancers.

Keywords TNF-α, CCL3, colorectal cancer

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List of abbreviations: APC = Adenomatous polyposis coli, CCL3 = C-C motif chemokine ligand3, ELISA = Enzyme-linked immunosorbent assay, HNPCC = Hereditary nonpolyposis colorectal carcinoma, IBD = Inflammatory bowel disease, Impα 3 = Importin alpha 3, NF-κB = Nuclear factor kappa-b, TME = Tumor microenvironment, TME = Tumor microenvironment, TNF-α =Tumor necrosis factor-alpha, WHO = World health organization

Introduction

The most frequent cancer in the gastrointestinal system and the fourth most common cause of cancer-related death worldwide is colorectal cancer (CRC). It is the primary cause of cancer-related morbidity and mortality in Iraq and ranks second in terms

of frequency of death after cardiovascular diseases [1].

With almost 1.9 million new cases, CRC is the third most commonly diagnosed cancer after lung and breast cancer. Of these, 72% develop in the colon, and only 28% start in the rectum. According to estimates, there could be a 60% increase in CRC incidence by 2030, and by 2040, there would be 3.2 million patients impacted by this tumor [2].

A pro-inflammatory cytokine tumor necrosis factor-alpha (TNF-α) is essential for the development of tumors [3]. Many tumor cells, such as B-cell lymphomas, effector T cells, breast cancer, pancreatic cancer, and colon cancer, release TNF-α. [4]. TNF-α is essential for the immune response and may play a role in the development of CRC. Cancer patients with elevated TNF-α expression levels, such as those with CRC, typically have a poor prognosis [5]. TNF-α is one of the many substances found in high concentrations in solid tumors and CRC patients' serum that have been related to the development of the spread of metastatic cancer [6].

Macrophage inflammatory protein-1α (MIP-1α) C-C motif chemokine ligand 3 (CCL3) is a member of the chemokine CC ligand family and is involved in the acute inflammatory state in the recruitment and activation of polymorphonuclear leukocytes through binding to the receptors CCR1, CCR4, and CCR5. It can be expressed on the surface of lymphocytes, epithelial cells, macrophages, and other cells facilitating immune cells' release of cytokines and encouraging the migration and aggregation of different types of cells [7].

Patients with CRC who had elevated serum levels of CCL3 and CCL4 had a worse prognosis than those who had normal levels. These results imply that blood levels of CCL3 and CCL4 may be predictive indicators for the prognosis of CRC patients and that a CCR5 inhibitor may offer the possibility of a novel therapeutic approach for the disease [8], Also, advanced disease stages and a poor prognosis have been associated with CRC's dysregulated expression of CCL3. Comprehending the function of CCL3 in CRC is crucial in deciphering the intricate relationship between inflammation, immune responses, and tumor growth [9].

This study aimed to evaluate the role of the serum level of both TNF-α and CCL3 in CRC patients compared to other colorectal conditions.

Methods

Patients and sampling

The College of Medicine, Al-Iraqi University has approved this study and the research proposal to be conducted in the presented form. None of the investigators and co-investigators participating in this study took part in the decision-making and voting procedure for this study. This Ethics committee is working by College of Medicine guidelines on biomedical research (The document number Ref. No. FM.S A/ 150 on May 5, 2024).

Two groups were made from 100 patients who suffer from gastro-intestinal tract problems:

- Colorectal cancer (CRC) group: 45 patients who were diagnosed definitely with CRC, before receiving any chemotherapy or radiation treatment (recent diagnosis).
- Colorectal disease (CRD) group: 45 patients who have other diseases in colorectal such as polyps, ulcers, IBD, Hirschsprung's disease, active inflammation, ulcerative colitis, and congestion. with ages ranging between (6-70) years old, of both sexes.

Blood samples were collected from different hospitals (the Medical City Hospital, Al-Amal Hospital, and Al-Bilad Private Hospital) from November 2023 to March 2024. The technical work was conducted at Qasr Al-Qadaa Health Center labs.

Five ml of blood was drawn from each group and put into a gel tube. Each gel tube was left for about 20 minutes at room temperature for clotting and later tubes were centrifuged for 20 minutes at 2000-3000 rpm. Then the serum per one gel tube was transferred into four Eppendorf tubes, each containing 500 µl and

stored at -20°C to be analyzed later. The hemolyzed samples were ten and they were discarded.

Enzyme-linked immunosorbent assay (ELISA) protocol

TNF-α and CCL3 ELISA kits (Elabscience Human TNF-α ELISA Kit /USA(E-EL-H0109) and CCL3 ELISA Kit/USA(E-CL-H0021) were used to determine their levels in both study groups and the results being recorded using a Mindray MR-96A Microplate- ELISA Reader system /Europe. Sandwich ELISA was employed according to the manufacturer's protocol.

Statistical analysis

Data were entered, checked, and analyzed using computer software programs of statistical package of social science (SPSS) version 26 and Statistica version 9.

Results

Demographic data

The age of the study samples ranged from 6 to 70 years old and its mean was 42.46±16.24, with most of the sample being in the age group of 40 to 59 years (46.7%). The mean age of CRC was 49.64±13.17 years and mostly was in the age group of 40-59 years old (53.3%), and the CRD was 35.27±15.95 years and mostly in the age group of 40-59 years old (40%), as shown in table (1).

Table 1. Characteristics of the study's sample (n=90)

Parameter		Study groups			P value
		CRC (n=45)	CRD (n=45)	Total (n=90)	
Age (years)	Mean±SD	49.64±13.17	35.27±5.95	42.46±16.24	<0.001[a]
	Range (min-max)	54 (16-70)	63 (6-69)	64 (6-70)	
Sex	Female	23 (51.1)	26 (57.8)	49 (54.4)	0.398[b]
	Male	22 (48.9)	19 (42.2)	41 (45.6)	

a: p value by unpaired t-test, b: p value by Chi-square test

Females were dominant among the CRC group (51.1% versus 48.9%) and in the CRD group (57.8% versus 42.2%) but it was non-significant differences (P >0.05).

The residency of the study's groups was distributed among several governorates, Baghdad was the most common place of residency for the study's groups of CRCs and CRD (33; 73% and 43; 96%) respectively followed by Diyala (4; 9% and 1; 2%), and Kirkuk (2; 5% and 1; 2%) (Figure 1).

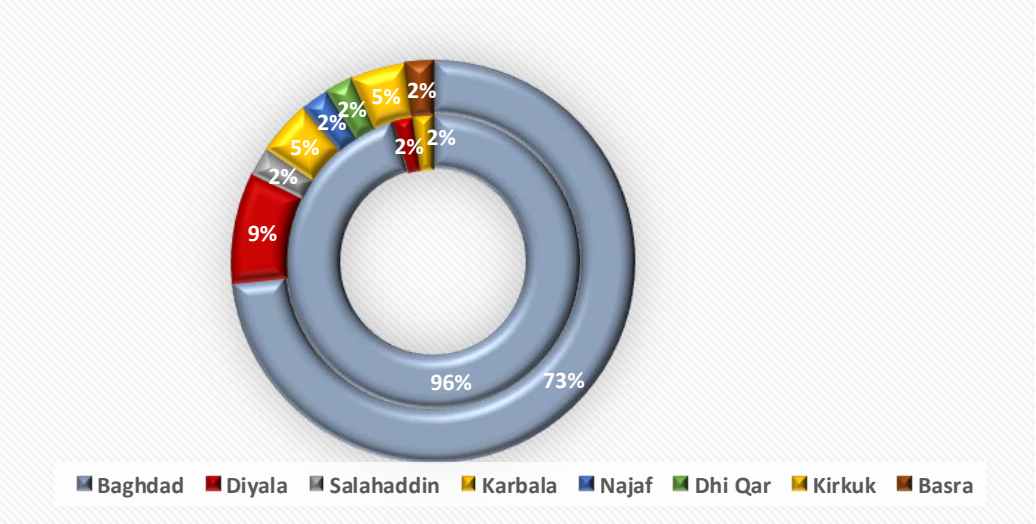

Figure 1. Distribution of residency among cases and control groups of study samples (n=90)

The immunological parameters

The parameters of TNF-α and CCL3 serum levels showed a significant difference (P <0.05) among the two study groups. The mean values of both TNF-α and CCL3 were significantly higher among the CRC group than the CR group (P <0.05) (Table 2), (Figures 2 and 3).

Table 2. Comparison of immunological parameters means between study's groups (n=90)

Immunological parameters	Cases (n=45) Mean±SD	Control (n=45) Mean±SD	P value
Tumor necrosis factor-alpha (TNF-α)	0.52±0.11	0.32±0.06	<0.001
C-C Motif Chemokine Ligand 3 (CCL3)	0.45±0.12	0.22±0.06	<0.001

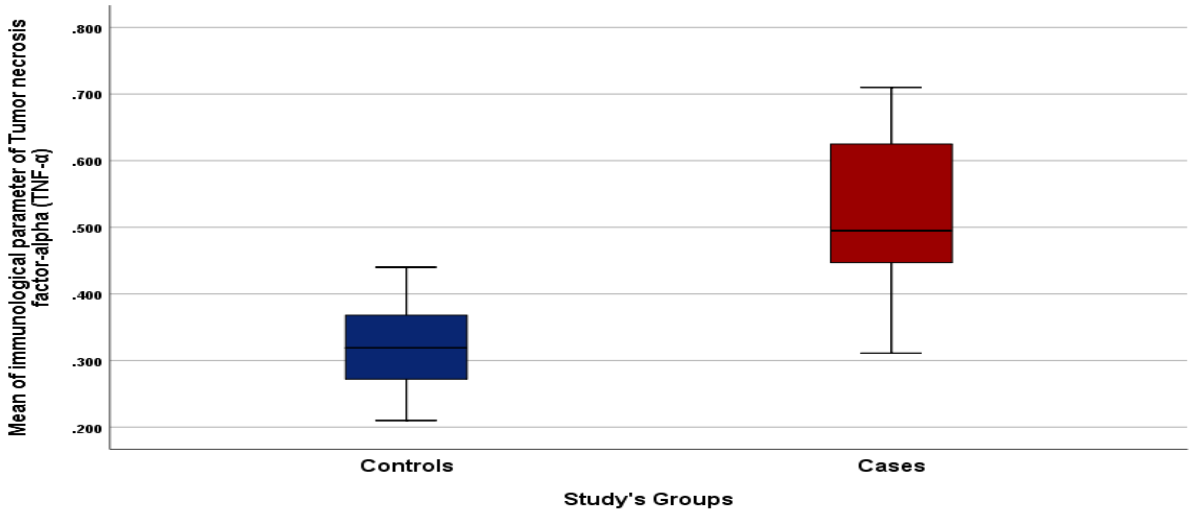

Figure 2. Comparison of immunological parameter of tumor necrosis factor alpha (TNF-α) among study's groups (n=90)

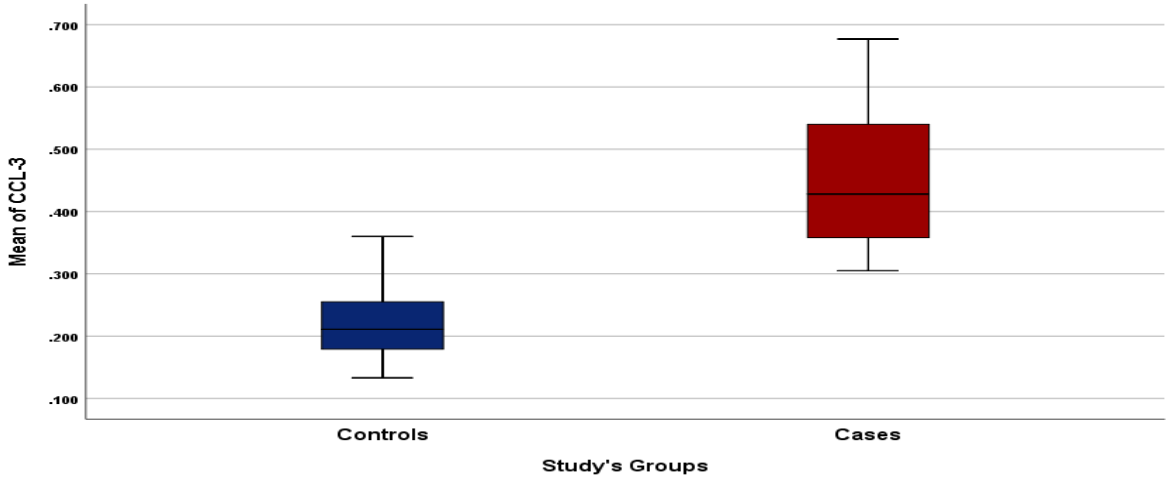

Figure 3. Comparison of immunological parameter of Chemokine (CCL-3) among study's groups (n=90)

The TNF-α is a predictive diagnostic marker for developing a risk of colorectal cancer, However, the optimal cutoff value of TNF-α for detecting patients with risk development of colorectal cancer was 0.37200 with a sensitivity of 88.9% and specificity of 80% and correctly predicted by the regression model of 87.8% with excellent area under the ROC curve (AUC) of 0.948 ± 0.021 (P=0.000) (Table 3) (Figure 4)

Table 3. Predictive value of TNF-α as a risk marker for developing colorectal cancer (n=90)

Parameter	Sensitivity	Specificity	Accuracy	Area Under The curve	P value
Tumor necrosis factor-alpha	88	80	87.8	0.948	0.000

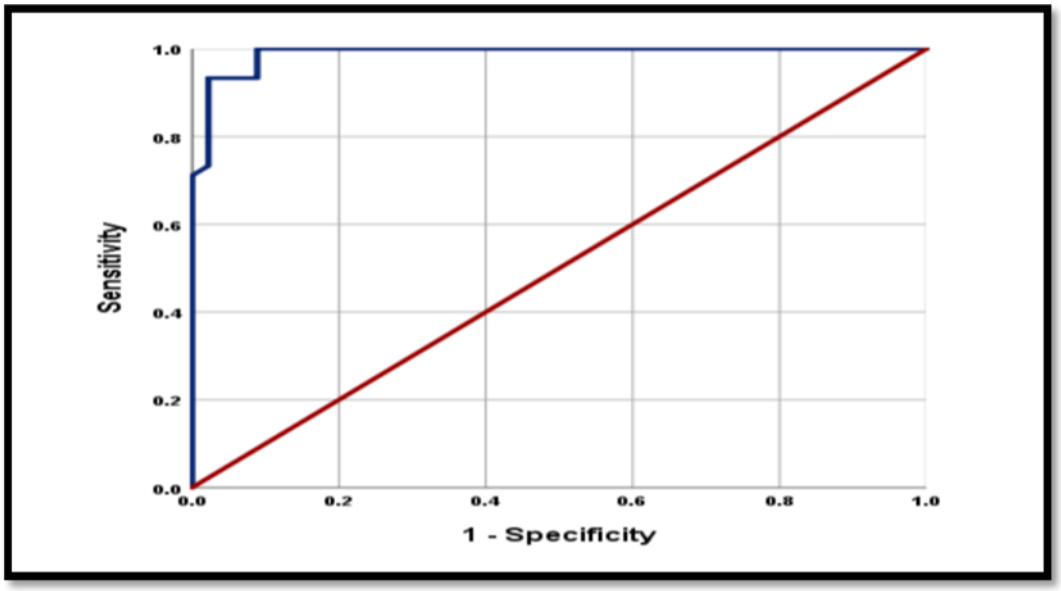

Figure 4. ROC Curve of colorectal cancer risk development predicted by the immunological parameter of TNF-α among study samples (n=90)

Similarly, the optimal cut-off value of chemokine (CCL-3) for detecting high-risk colorectal cancer development was 0.32500 with a sensitivity of 93.3%, and specificity of 97.8%, and correctly predicted by the regression model of 93.3% with excellent area under the ROC curve (AUC) of 0.989 ± 0.008 (P= 0.000) (Table 4) (Figure 5).

Table 4. Predictive value of Chemokine (CCL3) as a risk marker for developing colorectal cancer (n=90)

Parameter	Sensitivity	Specificity	Accuracy	Area Under The curve	P value
C-C Motif Chemokine Ligand 3	93.3	97.8	93.3	0.989	0.000

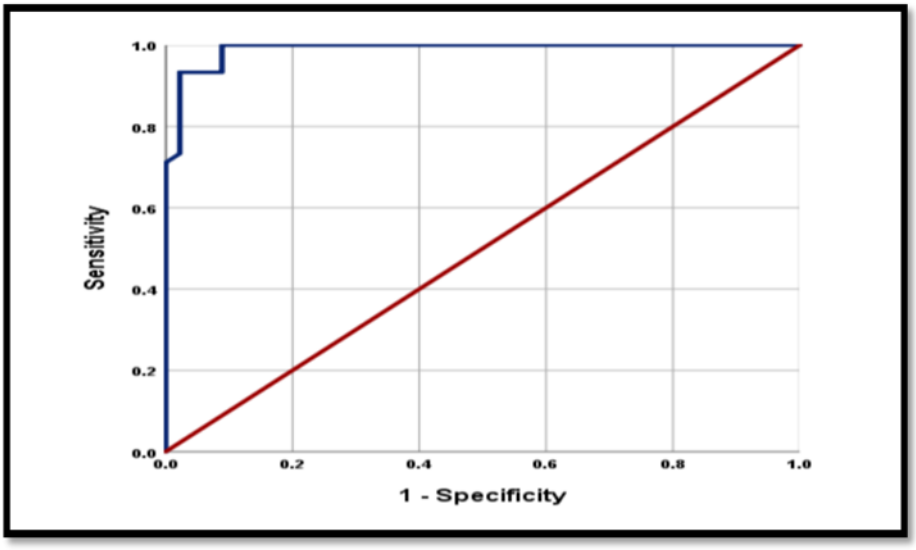

Figure 5. ROC Curve of colorectal cancer risk development predicted by the immunological parameter of Chemokine (CCL-3) among study samples (n=90)

Discussion

It's well understood that the complex interactions of aberrantly expressed cytokines, chemokines, growth factors, and matrix-remodeling enzymes promote CRC pathogenesis and evoke systemic responses that affect disease outcomes [10].

TNF-α is an important pro-inflammatory factor that plays a crucial role in the advancement and growth of CRC linked to colitis [11]. This inflammatory process is principally in charge of the NF-κB signaling pathway's persistent activation. Additionally, by causing DNA damage, it can increase the risk of CRC [12].

TNF-α and IL-6 are central players in developing CRC via activation of the critical oncogenic transcription factors nuclear factor Kappa-B (NF-κB) and signal transducer and activator of transcription 3 (STAT3), respectively [13,14]. IL-6 and TNF-α synergistically activate STAT3 and NF-κB to promote CRC cell growth in vivo [15]. TNF-α signaling has many components, including soluble or membrane-bound ligands and TNF receptors (TNF-αR1 and TNF-αR2). The binding of soluble TNF-α to TNF-αR1 activates NF-κB, a crucial transcription factor controlling many inflammatory pathways [16].

Increased serum IL-6 and TNF-α levels are associated with advanced-stage liver metastasis and short overall survival (OS) of patients with CRC. Furthermore, by upregulating inflammatory mediators such as TNF-α, TGF-β, chemokines, CXCL2, and SDF-1, IL-1β, CAFs induce CRC invasion and metastasis in vitro [17].

Other research has demonstrated that TNF-α plays a crucial function in epithelial cells and indirectly modulates the effects of ROS on the milieu surrounding stem cells. By changing the microbiota's makeup and activity, blocking TNF can lessen the risk of colorectal cancer [18]. Also, TNF-α is one of several factors that are highly expressed in solid tumors and CRC patients' serum and have been linked to the development of metastatic disease [19]. Furthermore, some researchers clarified that TNF-α overexpression in colorectal cancer is a poor prognostic factor. According to a study, TNF-α plays a key role in mediating the endothelial–mesenchymal transition (EMT) in the TME in CRC [20] which may be the cause of the high serum level of TNF-α in the CRC group compared to the CR group in this study.

Originally known as macrophage inflammatory protein 1α, CC chemokine ligand 3 (CCL3) is a proinflammatory cytokine that promotes

inflammation by inducing monocyte, T, and B cell chemotaxis [21]. According to a recent study, the TRAF6 and NF-κB pathway can stimulate the growth, invasion, and migration of colorectal cancer by positively correlating the expression of chemokine CCL3 and receptor CCR5 [22].

In murine models of colon cancer, it has been demonstrated that cancer-associated fibroblast (CAF) can be an important source of CCL3 [23].

A study showed that the metastasis of the main tumor to the colon is needed to increase the levels of circulating CCL3 and CCL4. Their systemic concentrations were the highest and lowest in CRC, respectively, in contrast to local expression [24].

In this study, it was found that CCL3 was significant at a high level in the patient's serum of CRC compared with the control in the result. In current study, the serological systems were utilized to investigate the levels of some immune parameters (TNF-α and CCL3) to see if they have high sensitivity and specificity for CRC, which can be considered predictive factors for the disease.

In conclusions TNF-α and CCL3 response was significantly high in the CRC group compared to the CRD group. In addition, cytokines; TNF- α, CCL3 could be used as a predictive test as one of the most important strategies for the diagnosis of CRC.

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Author contribution

Abdulhamid: conducted data collection, data analysis and wrote the manuscript. Dr. Abdullah: study design, assisted with manuscript revisions and statistical analysis, and approved the final product.

Conflict of interest

There is no conflict of interest stated by the authors.

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