



## Evaluating the levels of Tumor Necrosis Factor- $\alpha$ , Interleukin-6 and Vascular endothelial growth factor in patients with Colorectal Cancer

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### Abstract

Cytokines are polypeptides with several functions that are produced by a variety of bodily cells. They are clinically significant for illness diagnosis, treatment, and prevention. Interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- $\alpha$ ) are the most important cytokines for cell division and proliferation. Vascular endothelial growth factor (VEGF) is produced in excess in mesenchymal, epithelial, and especially in tumor cells. In this study the serum levels of IL-6, TNF- $\alpha$  and VEGF were detected for 41 colorectal cancer patients and 41 healthy control group at Nanakaly Hospital by comparing their serum concentrations for patients paining from colorectal carcinoma (CRC) with those of the control subjects. The result shows a significant increase in the cytokines TNF- $\alpha$ , IL-6 and VEGF in patients with CRC when compared with the healthy group. A significant positive correlation ( $p < 0.0001$ ) was found between TNF- $\alpha$  with IL-6 ( $r = 0.8200$ ) and VEGF ( $r = 0.7305$ ). According to this study, there is a direct correlation between CRC and blood levels of (TNF-a, IL-6, and VEGF). This suggests that these cytokines are crucial for the development and spread of colorectal cancer.

**Keywords:** Colorectal cancer, Tumor necrosis factor- $\alpha$ , Vascular endothelial growth factor, Interleukin-6, Cytokine.

## تقييم مستويات عامل نخر الورم- ألفا و إنترلوكين -6 وعامل نمو بطانة الأوعية الدموية في مرضى سرطان القولون والمستقيم

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### الخلاصة

السيبتوكينات هي بولي ببتيدات متعددة الوظائف يتم تصنيعها بواسطة خلايا الجسم المختلفة. لها أهمية سريرية من حيث تشخيص المرض ومعالجته وكيفية الوقاية منه. من أهم السيبتوكينات المهمة لتكاثر الخلايا وتمييزه هي عوامل نخر الورم ألفا وإنترلوكين-6. يتم إنتاج عامل النمو البطاني الوعائي بشكل زائد في اللحمية المتوسطة والظهارية، وخاصة في الخلايا السرطانية. في هذه الدراسة، تم الكشف عن مستوى إنترلوكين-6، وعامل نخر الورم ألفا، وعامل النمو البطاني الوعائي في امصال 41 مريضا مصابا بسرطان القولون والمستقيم و41 من مجموعة السيطرة في مستشفى نانكلي، من خلال مقارنة مستويات المصل للمرضى

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المصابين بسرطان القولون والمستقيم مع اشخاص الأصحاء. تم الحصول على علاقة إيجابية معنوية ( $P < 0.0001$ ) بين عامل نخر الورم ألفا مع إنترلوكين-6 ( $r = 0.8200$ ) وعامل النمو البطاني الوعائي ( $r = 0.7305$ ). من خلال هذه الدراسة تم الكشف على أن ارتفاع مستويات المصل (إنترلوكين-6 ، عامل نخر الورم ألفا ، وعامل النمو البطاني الوعائي) ترتبط ارتباطا وثيقا بسرطان القولون والمستقيم. والتي تشير إلى أن هذه السيتوكينات تلعب دورا حيويا في التسبب في الإصابة بسرطان القولون والمستقيم وتطوره.

## 1. Introduction

One of the most deadly cancers in the world and one of the main reasons for cancer-related death is colorectal cancer (CRC) [1, 2]. According to the most recent study on global cancer statistics, there were 935,000 deaths and two million new cases of colorectal cancer in 2020 [3]. There are a number of variables that have been linked to a significant rise in CRC incidence, including heavy alcohol and cigarette use, improper eating patterns, unhealthy lifestyle choices, and physical inactivity [4]. Inflammatory bowel disease (IBD), a chronic intestinal inflammation that is known to promote tumorigenesis in combination with genetic factors in the series of inflammation-dysplasia-cancer, including ulcerative colitis (UC) and Crohn's disease, are additional risk factors linked to the onset and progression of colorectal carcinoma [5, 6].

TNF- $\alpha$  is a pro-inflammatory cytokine that is mostly produced by macrophages and cancer cells [7, 8]. Cytokine ligand of the tumor necrosis factor family and TNF receptor superfamily have interacted with each other and the increasing of this interaction between TNF family and TNF receptor superfamily is one of the drivers of development in CRC. This system's activity could be one of the factors that cause CRC to progress [9]. Among its various biological activities are cell proliferation, differentiation, apoptosis, and inflammation. It is also a key molecule in the promotion of tumors that controls inflammatory processes. It has a range of impacts on cell activity via binding to certain high-affinity cell-surface receptors. TNF- $\alpha$  may promote cancer formation at low doses during the development of malignancies in addition to its apoptosis-inducing effects [10]. Numerous discoveries from studies suggest that TNF- $\alpha$  mediates a number of crucial tumor development processes, such as the activation of oncogenes, the breakdown of deoxyribonucleic acid, and metastatic dissemination. In CRC patients, TNF- $\alpha$  overexpression is highly correlated with tumor recurrence and positive lymph node metastases [11].

Interleukin-6 (IL-6) is a crucial pro-inflammatory cytokine that is also pleiotropic and has a number of biological effects, including the development of autoimmune and cancer illnesses. IL-6 may be generated by a variety of normal cells, including hematological, epithelial, muscle, and monocyte cells, as well as stromal cells. IL-6 plays a crucial role in inflammation, immunology, metabolism, reproduction, angiogenesis, and bone remodeling [12, 13]. According to Taniguchi and Karin [14], the presence of IL-6 in the blood was associated with tumor burden, a poor prognosis, and advanced stages of lung, mammary gland, kidney, and ovarian cancer.

Additionally, several studies have revealed that IL-6 is essential for the development and progression of CRC [15, 16]. It is evident that human colorectal cancer has elevated IL-6 levels [17]. Likewise, *in vitro* research has shown that IL-6 encourages the growth of colon cancer epithelial cells. Additionally, there is a startling lack of information on the association between IL-6 expression and clinic pathological features in human CRC [18].

Vascular endothelial growth factor (VEGF) is one of the most powerful angiogenesis stimuli. The development of new blood vessels is crucial to cancer development and metastasis. The VEGF is a multifunctional glycoprotein that has several effects on the vascular endothelium, including mitogenic, angiogenic, and vascular permeability [19]. When VEGF is overexpressed, mesenchymal cells, epithelial cells, and particularly carcinoma cells, such as breast and colon cancer, are affected [20]. The VEGF affects tumor development in a number of ways, including endothelial cell proliferation, neo-vascularization stimulation, and immune system destruction [21, 22].

In order to determine its correlation with the pathophysiology and development of CRC, this study examined the blood levels of TNF- $\alpha$ , IL-6, and VEGF in CRC patients.

## 2. Materials and Methods

This research was carried out in Erbil, Iraq, between July 2021 and January 2022. The samples consisted of 41 diagnosed individuals (aged (29-65)) with colorectal cancer at stages III and IV after receiving the first and second doses of chemotherapy. There were 21 male and 20 female patients. Additionally, a control group of 41 samples (21 men and 20 women) with ages ranging from 28 to 65 years old were taken from healthy volunteers. The work was completed in the province of Erbil's Nanakaly hospital. Samples are chosen at random. At several points during the study period, blood samples were taken from the cubital vein. To separate the serum from the samples, 5 ml of each sample was centrifuged at 6000 rpm for 10 minutes. Then, the serum samples were kept in a chiller at (-800 C), and storage samples were used to measure the levels of TNF- $\alpha$ , IL-6 and VEGF.

### 1.1 Measurements of TNF- $\alpha$ , IL-6 and VEGF

The TNF- $\alpha$ , IL-6, and VEGF serum levels of the malignancy and healthy groups were then assessed using an Enzyme-Linked Immunosorbent Assay (ELISA). The ELISA reader (BioTek TS 800) measures at 450 nm, the analytical results from the human ELISA kit (Sunlong). The TNF- $\alpha$ , IL-6, and VEGF values in the examined samples were measured in pg/mL.

## 3. Statistical analysis

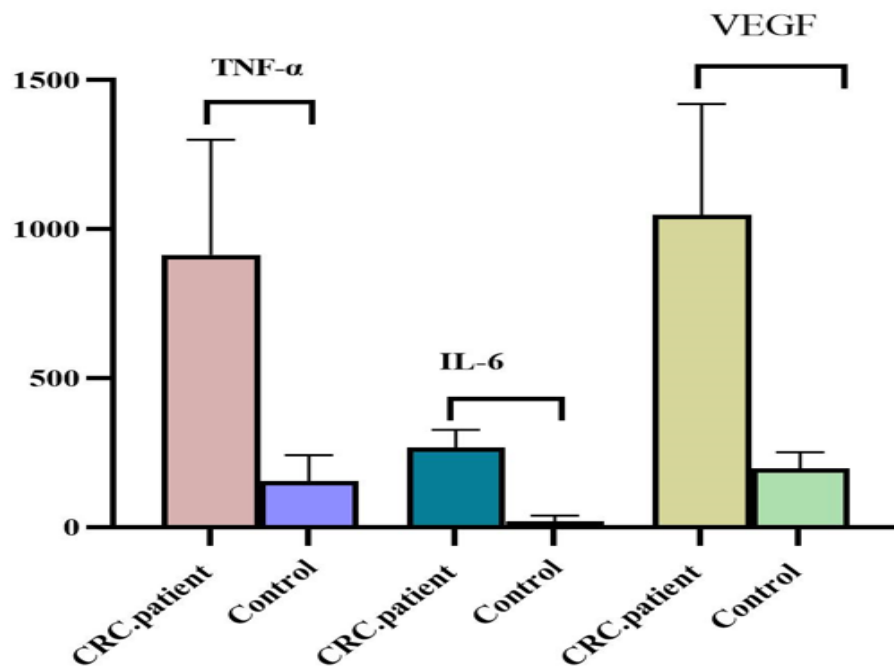
The statistical analysis was conducted using Graph Pad Prism 9.0. The data were presented using the mean  $\pm$  S.E. The T-test was applied to identify any differences between the two groups. Always using two-sided *P* values, significant differences were considered to be less than 0.05.

## 4. Results and Discussion

Eighty-two participants were used in the study, who were split into two groups (41 patients and 41 healthy controls). The findings of the blood levels of (TNF- $\alpha$ , IL-6, and VEGF) in patients and controls were shown in Table 1. When compared to the normal control groups (154.6 $\pm$ 13.67, 21.20 $\pm$ 2.884, and 197.8 $\pm$ 8.419), patients with CRC had statistically higher mean levels of TNF- $\alpha$ , (914.7 $\pm$ 60.25), IL-6 (267.2 $\pm$ 9.256), and VEGF (1048 $\pm$ 58.33). The present work provides additional data showing that the mean value of TNF- $\alpha$  was considerably greater in colorectal carcinoma cases than in healthy subjects. The present result was in agreement with that demonstrated by Csiszár *et al.* [23].

**Table 1:** Serum levels of (TNF-  $\alpha$ ), (IL-6), and (VEGF) in patients and normal healthy group

Serum parameters	CRC patient	Control	P-value
TNF- $\alpha$ (Pg/ml)	914.7 $\pm$ 60.25	154.6 $\pm$ 13.67	$p < 0.0001$
IL-6 (Pg/ml)	267.2 $\pm$ 9.256	21.20 $\pm$ 2.884	$p < 0.0001$
VEGF (Pg/ml)	1048 $\pm$ 58.33	197.8 $\pm$ 8.419	$p < 0.0001$

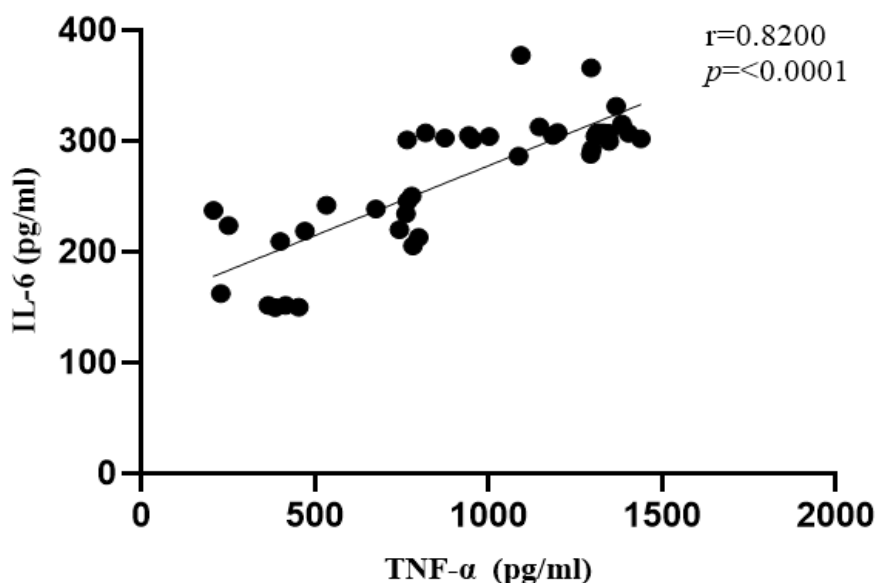
**Figure 1:** Concentration of TNF- $\alpha$ , IL-6, and VEGF (Pg/ml) in CRC patients and control subjects

The findings of this investigation were consistent with those of other earlier studies conducted by researchers from various nations, which demonstrated a considerable increase in total serum TNF- $\alpha$  levels with CRC [24, 25]. It might be related to ischaemia, tumor vascular inhibition, and hemorrhagic necrosis *in vivo*. By triggering an immune reaction against tumors, it also makes tumor cells more easily destroyed. To encourage tumor demise, TNF- $\alpha$  may interact with other cytokines [26]. TNF- $\alpha$  also has the ability to boost several other chemokines and cytokines. Protumorogenic actions include increased malignant cell survival, genetic mutations of cancer cells, and subsequent genetic alterations. Malignant cells can induce local angiogenesis and immunosuppression by combining myeloid cells with cytokines [11, 27]. Table 1 demonstrates a substantial difference in IL-6 levels between the patient and normal control groups. The similar outcome was observed in 2003, and the study revealed that the level of IL-6 in the blood of CRC patients was substantially greater than that of the control group. According to the study's results, colorectal cancer patients had considerably higher blood concentrations of IL-6 than healthy controls, which can be used as a disease marker to track progress [28]. It is believed that cancer cells secrete IL-6 to cause the elevated levels of IL-6 in cancer patients. The aforementioned notion was backed by the research of Chung *et al.* [29], who established that tumor cell lines and carcinoma tissue may both produce IL-6. Interleukin-6 has demonstrated to elevate tumorigenesis by autocrine or paracrine mechanism and had a suppressive impact on the immune system directed against the tumor [30]. The relation between tumor size with serum IL-6 concentrations and Dukes' stage may be described by previous

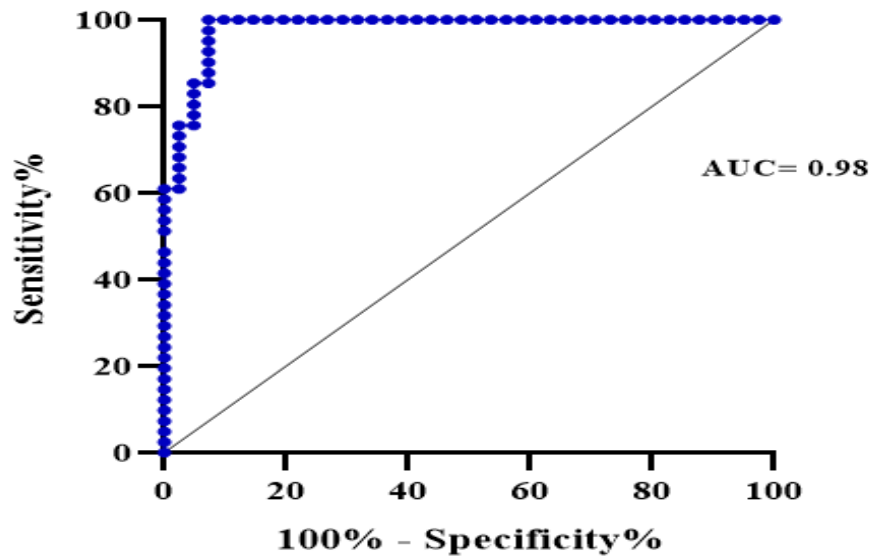
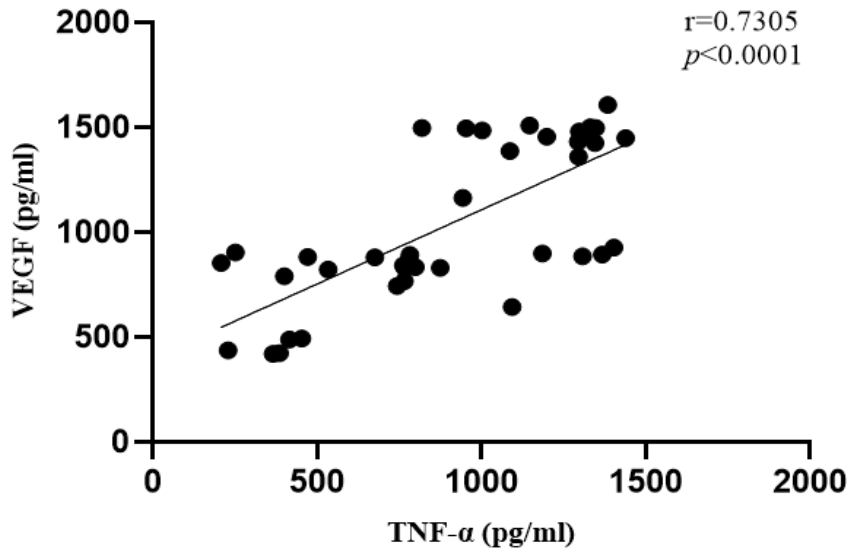
causes. Increase IL-6 levels were also importantly related to liver metastases[28]. This study was in the line with previous works [31, 32].

The degree of VEGF concentration, another parameter taken into account in those CRC patients, is displayed in Table 1 for both groups. The findings demonstrated that VEGF levels in CRC patients were considerably greater than in the control group. Previous research demonstrated that VEGF, which was higher in colorectal cancer [33, 34], was a critical factor in tumor angiogenesis. Lymph gland involvement, advanced stage, and poor survival have all been linked to angiogenesis and its evolving growth factors in a variety of tumor types [35]. The VEGF family of proteins and receptors is one of the key pathways connected with this process. Malignant cell lines have been shown to generate vascular endothelial growth factor in vitro, and in situ hybridization has been used to detect the presence of VEGF mRNA in a variety of human tumors, including the breast, gastrointestinal, and lung [36]. Both VEGF and its receptor are present in metastatic CRC at levels above normal. The degree of tumor vascularization may be determined using these two proteins [37, 38].

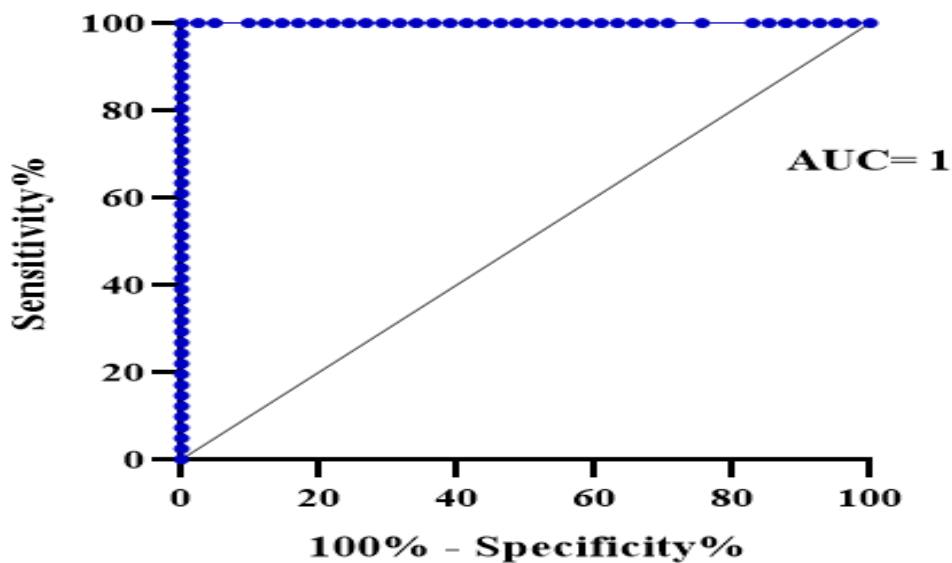
The correlation between TNF- $\alpha$  and IL-6 showed there are a positive correlation ( $r=0.8200$ ), which was statistically significant (Figure 2). Also, Figure 3 showed a statistically significant positive correlation between TNF- $\alpha$  and VEGF ( $r=0.7305$ ). Figures (4A, 4B, and 4C) display the receiver operating characteristic curve (ROC) curve of TNF- $\alpha$ , IL-6, and VEGF performance as a potential diagnostic marker for CRC. A relatively high AUC (area under the curve) suggests that testing for TNF- $\alpha$ , IL-6, and VEGF could be helpful in detecting CRC.



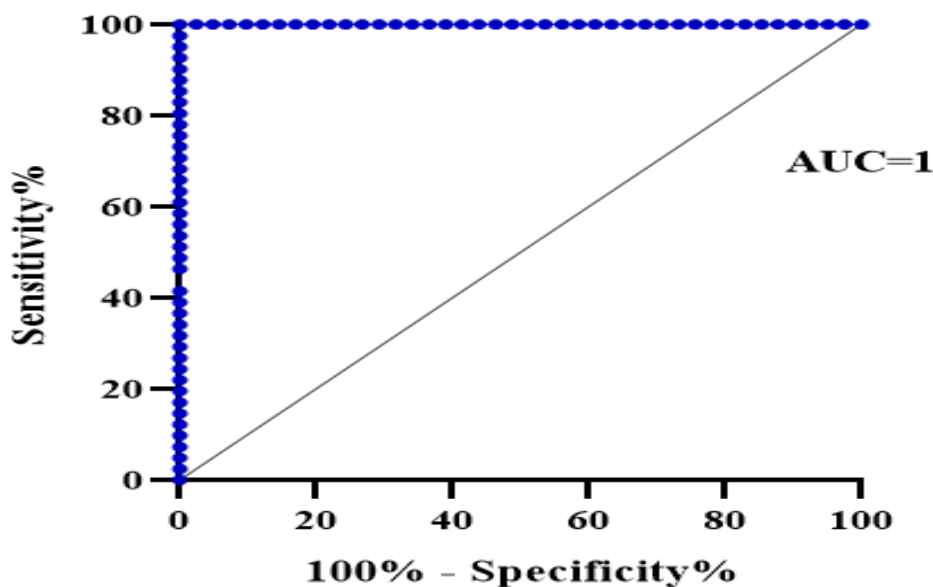
**Figure 2:** Shows the correlation between TNF- $\alpha$  and IL-6



**Figure 4A:** The ROC curve illustrates the sensitivity and specificity of TNF- $\alpha$  for the detection of CRC



**Figure 4B:** The ROC curve illustrates the sensitivity and specificity of IL-6 for the detection of CRC



**Figure 4C:** The ROC curve shows the sensitivity and specificity of VEGF for the detection of CRC

### Conclusion

According to the available data from this research, there were substantial differences in the levels of the cytokines TNF- $\alpha$ , IL-6, and VEGF, each of which were considerably higher in CRC patients than in healthy controls. As a result, these variables may be crucial in the development of colorectal cancer and serve as biomarkers for the disease.

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