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IL-35 and IL-10 Role in The Immune Response of Colorectal Cancer in Iraqi Patients

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ABSTRACT

Background: *Colorectal cancer (CRC) is presented in the colon and rectum (large intestine) and the disease is expanding worldwide, CRC ranks as the third most common cancer worldwide and second after breast cancer in locally. It is strongly linked to bad lifestyle factors. The study of the immunosuppressive status of CRC patients through screening and laboratory tests is crucial and is one of the priorities now. The objective of this study is to assess the immune response to colorectal cancer by measuring the serum concentrations of IL-35 and IL-10.* ***Methods:*** *The study has been approved by the College of Medical Al-Iraqia University. 100 male and female patients from the medical city and Al-kadhimiya hospitals suffering from problems in the colon and rectum participated in this study, they donated 5 ml of their blood which lasted for five months (from Aug. 2023 until Jun. 2024). The participants were divided into 2 groups: the 1st one contained CRC patients, while the 2nd contained Colorectal problems other than CRC.ELISA tests for IL-35 and IL-10 serum level estimation were used. Demographic data such as age, gender, address, and medical history were collected from the study participants.* ***Results:*** *The serum levels of IL-35 and IL-10 in CRC patients showed significant differences (P<0.05) when compared with the control group.* ***Conclusion:*** *IL-35 and IL-10 significantly appeared as indicators for the immune suppressive status of CRC patients.*

Key words: IL-35, IL-10, Colorectal Cancer.

Article Information

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INTRODUCTION

Colorectal cancer is a condition where cells in the colon, the large intestine, or the rectum, the passageway connecting the colon to the anus, become cancerous, be its growth out of control as a result of many causes leads to change in their DNA and It usually starts as benign polyps in glands to grow within the lining of the (colon, and rectum) and slowly develop into cancerous cells (Blanke, 2011).

The etiology of colorectal cancer (CRC) is not fully understood; however, it is associated with several factors, including genetics. Approximately 20% of CRC cases have a genetic component Among genetic syndromes that predispose to CRC are Familial Adenomatous Polyposis (FAP), the Mismatch Repair Gene (MMR), IN addition, bad environmental habits such as alcohol

consumption, smoking *obesity* Dietary factor*;* Current beliefs suggest that diets high in fat and animal protein, and low in cellulose, are associated with the incidence of colorectal cancer (CRC) (LI, R, 2022).

According to the World Health Organization in 2023, colorectal cancer ranks as the third most prevalent cancer globally, comprising about 10% of all cancer cases, and stands as the second leading cause of cancer-related mortality. It is estimated that in 2023, there were around 1.9 million new colorectal cancer cases and over 930,000 deaths from the disease worldwide (Roshandel G,2024) In Iraq, colorectal cancer (CRC) ranks as the second most common cancer (Iraqi cancer registry ,2022). Furthermore, CRC is among the most lethal cancers; the combined incidence rate for men and women has risen from 2.28 to 6.18 per 100,000 people between 2000 and 2019, corresponding to an annual percentage increase of 5.11% (Clancy,2023). Studies indicate that early diagnosis is crucial in reducing the incidence and mortality rates of colorectal cancer (CRC), as it often progresses from preexisting adenomatous polyps in the colon or rectum to a malignant tumor. Laboratory tests that measure key immunological parameters, including IL-35 and IL-10, are significant diagnostic methods due to their high sensitivity and specificity in detecting CRC.

Recent research has underscored the importance of IL-35 and IL-10 in colorectal cancer (CRC). IL-10, known for its anti-inflammatory properties, is a pleiotropic cytokine that can promote or inhibit tumors. It plays a vital role in regulating inflammation and maintaining cellular homeostasis. Primarily, it serves as an anti-inflammatory cytokine, safeguarding the body against an uncontrolled immune response (Hamad,2021). IL-10 plays a role in CRC by influencing the mucosal immune response and contributing to colorectal carcinogenesis and tumor microenvironment (TME) in CRC. The cytokine network between cancer cells and the TME, can regulate inflammatory processes in colorectal cancer (CARLINI ,2023).IL-10 is expressed by macrophages ,T lymphocytes and NK cell infiltrating colorectal cancer metastasis (CRLM) and acts as an immunosuppressive cytokine. Blocking IL-10 leads to increased tumor apoptosis and enhances immune responses, including increased CD8+ T cell frequency and IFNγ expression, suggesting that IL-10 is a mechanism of immune evasion (Farc,2023). In the small intestine, IL-10 expression by CD4+ T cells is protective and required for immune surveillance, as loss of IL-10 leads to impaired cytotoxic activity and progression to cancer.

IL-10 has potential as a biomarker for the non-invasive diagnosis of colorectal cancer. High expression of IL-10 is associated with a worse prognosis in colorectal cancer patients (Kim,2022). Interleukin-35 (IL-35) is a cytokine belonging to the IL-12 family, known for its immunosuppressive properties IL-35 is secreted by regulatory T (Treg) cells and B cells, playing a crucial role in immune modulation in various diseases like autoimmune conditions, cancer, and infectious diseases (Hu,2021).

As well as IL-35 plays a significant role in colorectal cancer by exerting both inhibitory and stimulatory effects. Research indicates that IL-35 expression is minimal in colon cancer tissues but significantly higher in surrounding normal tissues. The reduced expression of IL-35 is linked with the advancement of cancer and a decrease in patient survival rates (LI &P.,2023). Furthermore, IL-35 suppresses β-catenin, which inhibits colon cancer cell migration, invasion, proliferation, and colony formation, acting as a potential therapeutic target

(Liu,2021). On the other hand, IL-35 secreted by tumor cells promotes tumor growth and metastasis by modulating the tumor microenvironment, enhancing angiogenesis, and reducing CD8+ T cell infiltration (ALmutairi,2022). Therefore, IL-35 emerges as a dual player in colorectal cancer, influencing both tumor progression and anti-tumor immune responses.The study is designed to assess the role of IL-35 and IL-10 through serum level measurements in patients with colorectal cancer (CRC) in comparison to individuals with other colorectal conditions.

MATERIAL AND METHODS

Patients and Sampling:

The study has been approved by the College of Medical Al-Iraqia University. Two groups were made from 90 patients who suffer from GIT problems: the CRC group (45) patients who were diagnosed definitely with (CRC) and (CRD) group (45) patients who have other diseases in colorectum such as polyps, ulcers, IBD, HIRSH-springs disease, Active inflammation &congestion, etc.) 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 Hospital) from Aug. 2023 until Jun. 2024. The technical work was conducted at Qasr Al-Qadaa Health Centre 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 2Eppendorf tubes each containing 500 µl and stored at - 20 ℃ to be analyzed later. The Hemolyzed samples were ten and they were discarded.

Eliza protocol

Two serological tests were performed (IL-35 and IL-10) to determine their levels in CRC patients been done by using Elabscience / Human IL-35 ELISA Kit /USA(catalogue number E-EL-H2443), as well as IL-10 we used Elabscience Human IL-10 ELISA Kit /USA(E-EL-H6154) with the results being recorded using a MindrayMR-96A Microplate- ELISA Reader system /Europe. We employed Sandwich ELISA in accordance with the manufacturer's protocol. Sensitivity and specificity for the manufacturer of the assay are 91.1% and 97.8%, respectively.

Statical analysis

Data entry, verification, and analysis were conducted using the Statistical Package for the Social Sciences (SPSS) version 26 and STATISTICA version 9 software programs.

RESULTS

Demographic data:

The age of the study samples ranged from 6 to 70 years old and its mean was 42.46 ± 16.237, With the majority of the sample falling within the 40 to 59 years age group. (46.7%). The mean age of CRC was 49.64 ± 13.165 years and mostly was in the age group of 40-59 years old (53.3%), and the CRD was 35.27 ± 15.945 years and mostly in the age group of 40-59 years old (40%), as in table (1)

Females were dominant among the CRC group (51.1% vs. 48.9%) and in the CR group (57.8% vs. 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 CRC and CR (33; 73% and 43; 96%) respectively followed by Diyala (4; 9% and 1; 2%), and Kirkuk (2; 5% and 1; 2%), (Figure 1).

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

Characteristics	Study groups			
	CRC(n=45)	CRD (n=45)	Total (n=90)	Significance
Age (years)				
Mean ± SD	49.64 ± 13.165	35.27 ± 15.945	42.46 ± 16.237	t= -4.664, df: 88, P= 0.000[a]
Range (min-max)	54 (16- 70)	63 (6- 69)	64 (6- 70)	
Sex				
Female	23 (51.1)	26 (57.8)	49 (54.4)	X^2= : 0.714, df:1, P = 0.398[b]
Male	22 (48.9)	19 (42.2)	41 (45.6)	

a: Unpaired T-Test, b: Chi-Square Test.

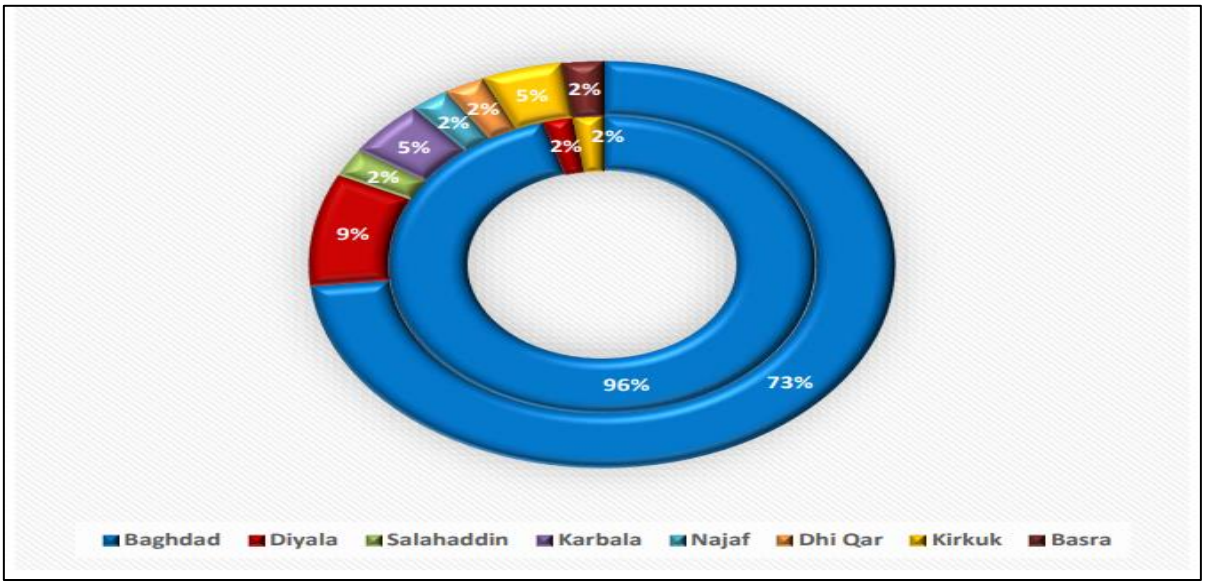

Figure 1. Distribution of residency among cases and control groups of study samples.

The study parameters

IL-35 and IL-10 serum levels were checked by ELISA in both CRC and CR groups. The Kolmogorov-Smirnov and Shapiro-Wilk tests are utilized for analyzing data where the mean serum levels are measured. of IL-35 and IL-10 were higher significantly (P > 0.05) among CRC group than that of CR group (Table 3-1), (Figure 2) and (Figure3). The optimal cutoff value of Interlukine-35 (IL-35) for detecting high risk of colorectal cancer development was 0.26800 with a sensitivity of 91.1%, and specificity of 97.8% and correctly predicted by the regression model of 95.6% with excellent area under the ROC curve (AUC) of 0.993 ± 0.005 (*P*= 0.000) (Table 4-5) (Figure 3). Among a 90-study samples, the optimal cutoff value of interlukine-10 (IL-10) for detect patients with high risk of development of colorectal cancer was 0.17400 with a sensitivity of 100%, and specificity of 95.7% that correctly predicted by the regression model of 100% with excellent area under the ROC curve (AUC) of 1.000 ± 0.000 (*P*= 0.000) (Table 5) (Figure 5).

Table 2 Means' comparison of immunological parameters among study's groups (n=90):

Immunological Parameters (Mean ± SD)	Study groups (n=90)		Mean differences	Significance [a]
	Cases (n=45)	CRD (n=45)		
Interlukin-10 (IL-6)	0.33571 ± 0.030861	0.13893 ± 0.22020	-0.196778	*t*= -34.819, df:88, *P*= 0.000
Interlukin-35 (IL-35)	0.30060 ± 0.027354	0.17513 ± 0.042670	-0.125467	*t*= -16.606, df:88, *P*= 0.000

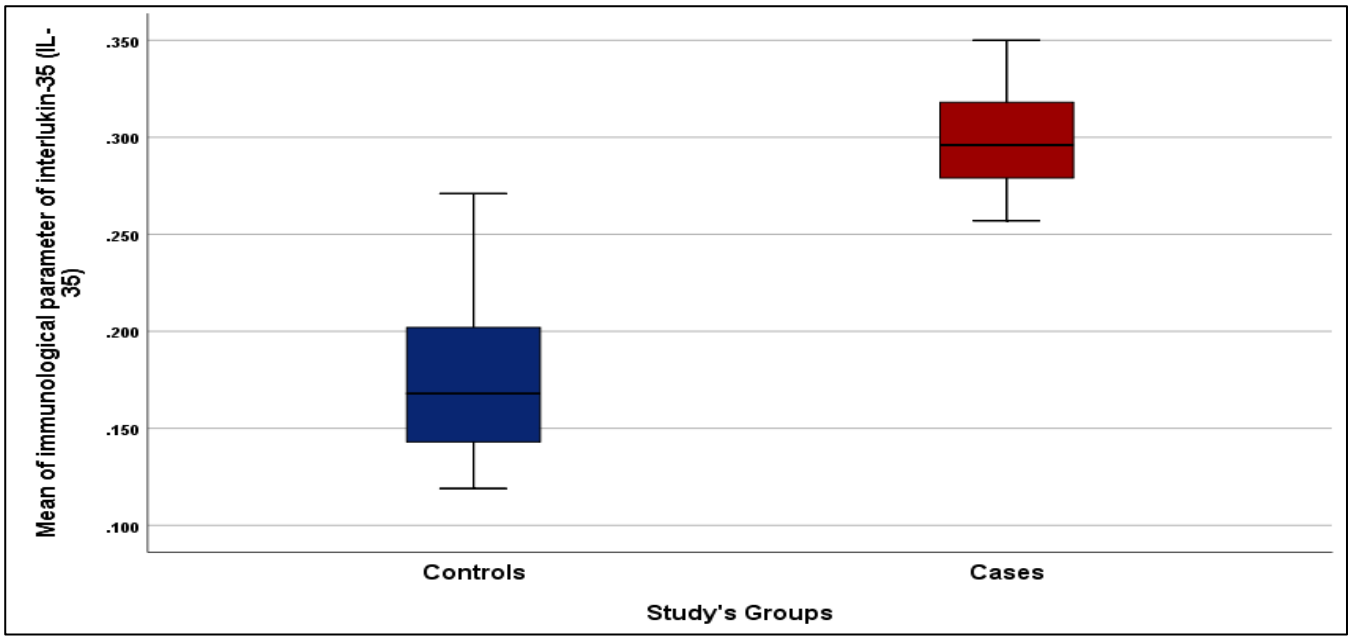

Figure 2 Comparison of immunological parameter of interlukin-35 (IL-35) among study's groups (n=90).

Table 3 Predictive value of interlukine-35 (IL-35) as a marker for developing colorectal cancer (n=90).

Parameter	Validity of model				
	Sensitivity (Sn)	Specificity (Sp)	Accuracy	Area Under the curve (AUC)	Significance (*P*-value)
Interlukine-35 (IL-35)	91.1	97.8	95.6	0.993	0.000

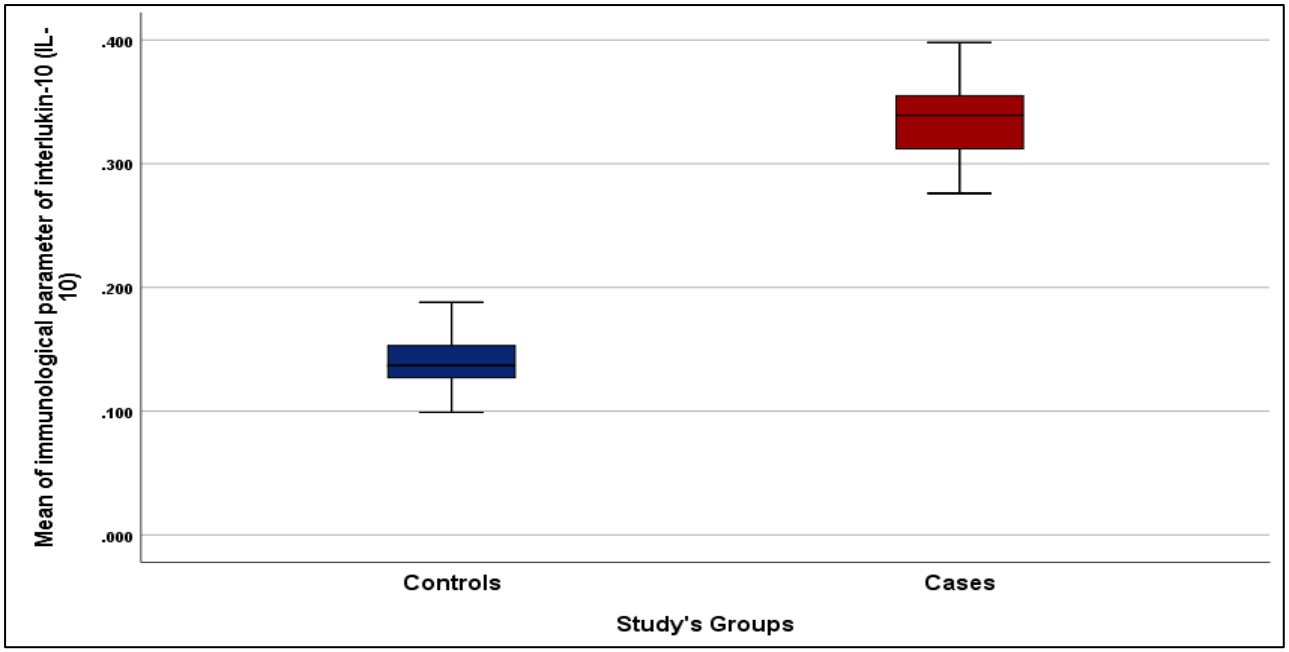

Figure 3 Comparison of immunological parameter of interlukin-10 (IL-10) among study's groups (n=90).

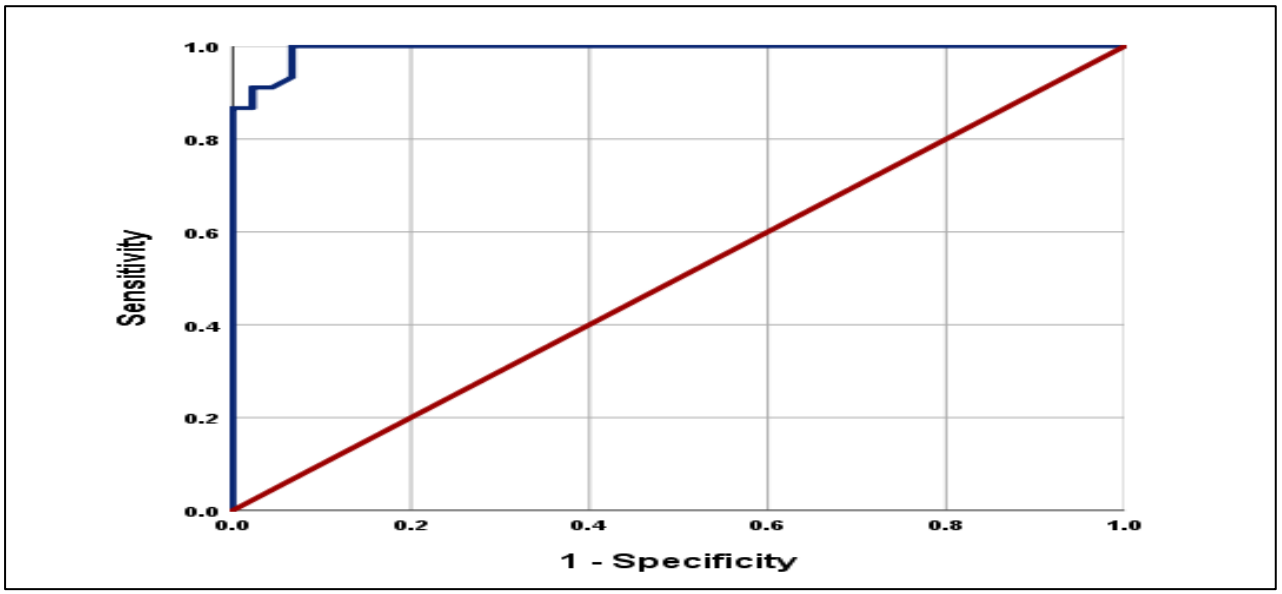

Figure 4 ROC Curve of colorectal cancer risk development predicted by immunological parameter of interleukin-35 (IL-35) among study samples (n=90).

Table 4 Predective value of interlukine-10 (IL-10) as a marker for developing colorectal cancer (n=90).

Paramter	Validity of model				
	Sensitvity (Sn)	Specificity (Sp)	Accuracy	Area Under the curve (AUC)	Significancy (*P*-value)
Interlukine-10 (IL-10)	100	95.6	100	1.00	0.000

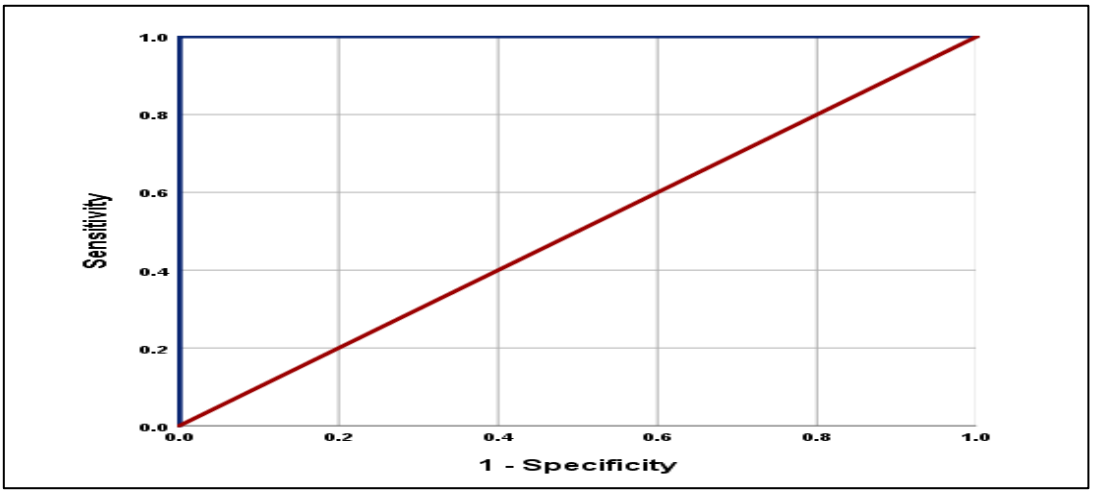

Figure 5 ROC Curve of colorectal cancer risk development predicted by immunological parameter of interleukin-10 (IL-10) among study samples (n=90).

DISCUSSION

Colorectal cancer is a diverse disease influenced by a buildup of genetic and epigenetic alterations, which transform normal colonic mucosa into an aggressive form of cancer (Osuna-gómez,2023; Bulliard,2023). It ranks among the third most common cancers globally, with significant mortality rates, especially in individuals over 50 years old (Huang,2024), and locally it is considered third largest among cancers and the fourth leading cause of death. In Iraq, colorectal cancer (CRC) remains predominantly a disease affecting the elderly, with rising incidence and mortality rates observed across all age groups and genders. This trend indicates a pressing need for a reassessment of health policies concerning CRC **(**Kim,2022). The thing this requires finding diagnostic methods that contribute to reduce the chances of some digestive system diseases turning into cancers. Perhaps the most important of these is considering some of the immune parameters that are affected and well be high specificity and sensitivity as (IL-35 and IL-10) in these diseases as prognostic factors for some GIT cancers such as (colon, and rectum) cancer, If it is diagnosed early.

In our current study, we utilized the serological systems to investigate the levels of some immune parameters (IL-35, IL-10) to see if they have high sensitivity and specificity for colorectal cancers, which can be considered as predictive factors for the disease. Proven some studies elevated levels of IL-35 have been associated with colorectal cancer (CRC) progression (CHANG,2020). Research indicates that in CRC patients, there is an increase in various inflammatory cytokines, including IL-35, in the peripheral blood compared to healthy individuals (Zeng,2013). Furthermore, studies have shown that IL-35

plays a role in promoting cell proliferation, migration, and invasion in CRC cells, contributing to tumor growth and metastasis (Mirlekar,2022).The upregulation of IL-35 has been linked to poor prognosis in CRC, highlighting its potential as a biomarker for disease severity and a therapeutic target .These findings suggest that IL-35 may serve as a crucial factor in the immunoregulation and progression of colorectal cancer, emphasizing its significance in the context of CRC research(Saeed,2020 ; LI, P.,2024) Research from multiple studies has consistently demonstrated a decrease in serum IL-35 levels in patients with colorectal cancer. The reduction of serum IL-35 levels following surgical resection suggests a potential role for IL-35 in the progression of colorectal cancer, which is associated with a poor prognosis. Collectively, these findings indicate that IL-35 could be a valuable biomarker for evaluating the progression and prognosis of colorectal cancer in clinical settings (Chang, 2020; Yazdani,2020).

Studies have shown conflicting results regarding the serum levels of interleukin-10 (IL-10) in colorectal cancer (CRC) patients. While some studies have reported a significant increase in IL-10 levels in CRC patients compared to healthy controls (Kim ,2022), others have indicated a decrease in IL-10 levels after chemotherapy treatment in metastatic CRC patients, with lower levels associated with improved overall survival (Mohammadpoor, 2021). Additionally, a study focusing on miRNA-221 and IL-10 in CRC patients found that higher levels of miRNA-221 and IL-10 were correlated with a worse prognosis, suggesting a potential role as prognostic markers. These findings highlight the complexity of IL-10's role in CRC and the need for further research to elucidate its significance in the disease (Hamad,2021).

Our study indicates an increase in IL-35 and IL-10 levels in patients with colorectal cancer (CRC),and may be they have a direct relationship in CRC where in CRC ,IL-35 act as a pro-tumor cytokine ,where its height is related with severity and staging of CRC , It can convert immune cells into a tumor growth-promoting phenotype and facilitate tumor angiogenesis ,And may be lead to high levels of IL-10 which contributed cancer pathogenesis and prognosis indicating its potential as a prognostic marker for detecting CRC patients' prognosis. The expression levels of IL-10 in CRC patients vary based on different stages, tumor sizes, histological grades, and situations of distant metastasis, with a gradual decrease post-operation. Higher levels of IL-10 in CRC patients have been associated with increased cancer recurrence rates, also suggesting that IL-10 can serve as an indicator of CRC prognosis (Huang,2024).

CONCLUSION

Serum levels of IL-35 and IL-10 were significantly elevated in the colorectal cancer (CRC) group compared to the control group, suggesting their potential as predictive markers for CRC.

Ethical approval

The present study Which is conducted by authors (Saja j. Mohammed, Ahmed Rushdi Abdullah and Nawar Sahib Khalil) was approved by the local Department of AL-Iraqia University, College of Medicine and medical city of committee.

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