Original Research Article

Correlation between IL-10 as Cancer Biomarker and some Characteristics of Colorectal Cancer Patients

Abstract

Colorectal cancer (CRC) is classified as one of the most prevalent cancer types with high morbidity and mortality rates worldwide. Patients of CRC have been shown to express a detectable cytokine in serum which contributes to cancer pathogenesis. Therefore, the level of serum interleukin 10 (IL-10) in CRC patients was investigated in this study. Patients' medical records with CRC admitted to the Rizgary and Nanakali hospitals, Erbil, Iraq was analyzed as the study group compared to the healthy volunteers' control group. Seventy-one serum samples were collected, thirty-one from diagnosed CRC patients and forty from healthy controls. The concentrations of IL-10 in the sera were assessed by enzyme-linked immunosorbent assay (ELISA). The present finding showed that IL-10 Was significantly elevated in CRC patients' sera compared to the control group, suggesting its usefulness for detecting CRC patients' prognosis. A non-significant Pearson correlation was detected between IL-10 serum levels and the CRC group's age, gender, and body mass index. Herein is the first study on the evaluation of IL-10 levels in CRC patients in Kurdistan, Iraq.

Keyword: Colorectal cancer, Interleukin-10, ELISA, Biomarker, Cytokines

Introduction

Cancer is a worldwide health problem, mainly in developing countries. Globally, GLOBOCAN recorded 18.1 million cancer cases, and 9.6 million deaths resulted from cancer in 2018. It is a significant reason for morbidity and mortality worldwide, regardless of the human development

status [1]. Colorectal cancer (CRC) is categorized as the 3rd most frequently identified cancer type and the 4th reason for cancer deaths globally. Its impact is predicted to rise 60% to about 2.2 million new cases and 1.1 million deaths through 2030 [2]. Despite the progress in diagnostic and therapeutic methods, CRC remains a significant health problem with high morbidity and mortality [3]. CRC is predominant and now responsible for about 10% of cancer-related mortality in western countries. Its increase in developed countries can be related to aging people, unfavorable dietary habits, and increased risk influences such as smoking, little physical exercise, and obesity [4].

Regarding the trend of gastrointestinal cancers in Iraq, stomach and Colorectal cancer showed a significant increase after 2007 ($p < 0.001$), while females and males were equally affected. [5]. In a recent study, Shnawa and Al-Majmaie pointed to the remarkable growth in cancer research in Iraq, mainly after the 2000s, reflecting the rising cancer incidence [6].

Interleukin 10 (IL-10), a potent anti-inflammatory cytokine, is mainly generated by Th2 cells, macrophages, B lymphocytes, and keratinocytes in usual conditions. Nevertheless, an abnormal increase in its expression happens during tumorigenesis. In a cancer patient's body, the Th2 cells, the primary source of IL-10, increase abnormally. Also, regulatory T cells secrete a high concentration of IL-10 and few amounts of IL-2. As well, tumors by themselves may produce IL-10, resulting in an increased quantity of IL-10 [7]. IL-10 is an anti-inflammatory cytokine controlling innate and adaptive immune responses [8]. It may cause stimulation of prostate cancer's pathogenesis by inhibiting the secretion of pro-inflammatory cytokines, like TNF-α, IL-6, and IL-8, and preventing tumor invasion and metastasis by indirect mechanisms. Also, they mentioned that genetic variation in IL10 and probably *TLR4* is related to prostate cancer risk. [8,9]. In recent years, many studies focused on the emergent importance of serum IL-10 as a diagnostic and prognostic marker for CRC patients [10,11,12]. But still no more updating and precise information available regarding this issue in Iraq. Therefore, this work was investigated the serum level of IL-10 of CRC patients and clarified its association with some demographic parameters.

Materials and methods

Study subjects

Colorectal cancer (CRC) patients of different ages and from both sexes are eligible for this study, compared to healthy subjects as a control group.

Sample's selection and collection

Peripheral blood samples were taken from thirty-one post-diagnosed patients with colorectal cancer (CRC) from both Rizgary and Nanakali hospitals from February to November of 2020. Associated hospital staff, doctor, and patient permissions were obtained before taking the samples. For healthy controls (H.C), peripheral blood samples from forty age-matched individuals were randomly selected from healthy people of Soran city. A mount of 3 ml of whole peripheral blood was taken from the study subjects and placed in serum separation and EDTA tubes. All samples were preserved in a cooling container and transferred to the molecular biology research laboratory, Department of Biology/ Faculty of Science/ Soran University.

All methods performed in the present study, including participants, followed the ethical standards of the national research committee.

Serum separation

The samples were allowed to clot for half an hour at room temperature before centrifugation. The blood was centrifuged for 15 minutes at 3000 ×g. From every sample, three aliquots of 0.7 ml of serum were prepared. The sera samples were stored at -20°C until further analysis. The sera of CRC patients and H.C were used to detect interleukin 10 (IL-10) by ELISA.

Estimation of serum IL-10 level by ELISA

The serum level of interleukin 10 (IL-10) was assessed by enzyme-linked immunosorbent assay (ELISA) as described previously [13]

Human Interleukin 10 (IL-10) ELISA Kit based on biotin double antibody sandwich technology (Wuhan Hi-tech Medical Devices Park, CUSABIO/ China, Catalog Number. CSB-E04593h) was used. Diluted Biotin-antibody, Diluted HRP-avidin, and Standard solutions were prepared and processed according to the manufacturer's instruction of the kit.

Briefly, 100µl of standard and serum was placed into each well of ELISA plate and incubated for 2 hr. at 37°C according to the assay kit protocol. The plates were incubated with 100µl of Biotin-

antibody antibody (1X) at 37°C for 1hour. After some washes, the plates were incubated with 100µl of HRP-avidin (horseradish peroxidase solution) for 1 hr at 37°C.

A mount of 90µl of TMB Substrate was added to each well and incubated for 15-30 minutes at 37°C in dark conditions. Finally, an aliquot of 50µl of stop solution was added to each well gently, and color-changing was observed. Each well's optical density (O.D) was measured within 5 minutes using a microplate reader at 450 nm wavelength. The ELISA standard curve was drawn by plotting the O.D. values on the Y-axis and calibration concentrations (I.U./ml) on the X-axis. A standard curve was prepared to evaluate the concentration of tested samples (Figure 1).

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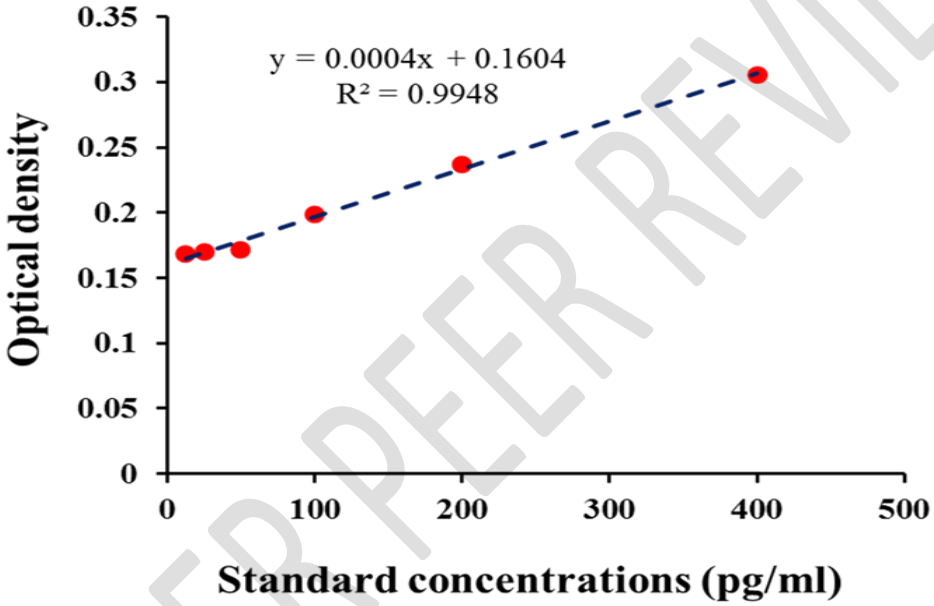

Figure1. The ELISA standard curve of IL-10. It was created by plotting the optical density (wavelength 450 nm) on the y-axis and the standard concentrations on the x-axis. The concentration of IL-10 in the study subjects was estimated using the slope equation.

Statistical analysis

The data analyzed statistically used a normality test at the beginning followed by a non-parametric test (Mann-Whitney Confidence Interval). All statistics and other graphics were performed using Graph Pad Prism 7.05 software. The test is significant at <0.05. Pearson correlation was used for assessing the correlations between IL-10 serum level and some study subjects' parameters like gender, age, and body mass index.

Results

1.1. Study subject demography

Samples were collected and analyzed from 31 colorectal cancer (CRC) patients and 40 healthy controls (H.C.) regarding sex, age, and Body Mass Index (BMI), as shown in Tables 1,2 and 3.

Table1. Distribution of study subjects according to gender.

Groups	Females (%)	Males (%)	Total
Colorectal cancer	14	17	31
Healthy controls	23	17	40
Total	37	34	71

Table2. Distribution of study subjects according to age groups.

Groups	G1(< 20 y)	G2(20-29y)	G3(30-39y)	G4(40-49y)	G5(50-59y)	G6(60-70y)	Total
Colorectal cancer	0	0	6	3	12	10	31
Healthy controls	5	6	10	9	6	4	40
Total	5	6	16	12	18	14	71

Table3. Distribution of the CRC patients according to body mass index.

Groups	Normal weight	Overweight	Obesity

Colorectal cancer patients	**9**	**16**	**6**

1.2. Estimation of serum IL-10 level

The results showed a significant increase in IL-10 level in the CRC group compared to H.C. ($p<0.05$), the means were 497.97 ± 77.0 and 38.06±43.91 pg/ml, respectively. IL-10 estimation revealed significantly raised sensitivity for distinguishing between CRC patients and healthy controls, as illustrated in Figure 2.

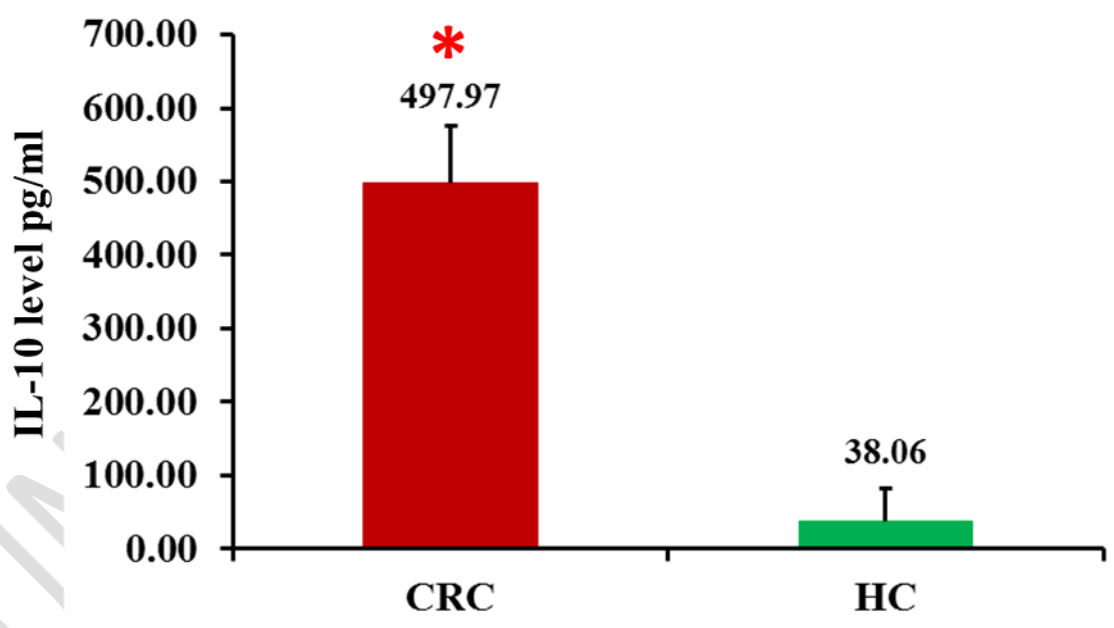

Figure2. The level of IL-10 in CRC and the control group. The level of IL-10 was significantly increased in CRC patients compared to H.C. (497.97 ± 77.0 and 38.06±43.91 pg/ml, respectively) measured by ELIS test results ($P<0.05$).

1.3. Serum level of IL-10 according to gender

The current results showed a significant elevation in serum IL-10 level in the CRC group compared to the H.C. group according to gender with statistical significance ($p<0.05$)., as shown in Figure3.

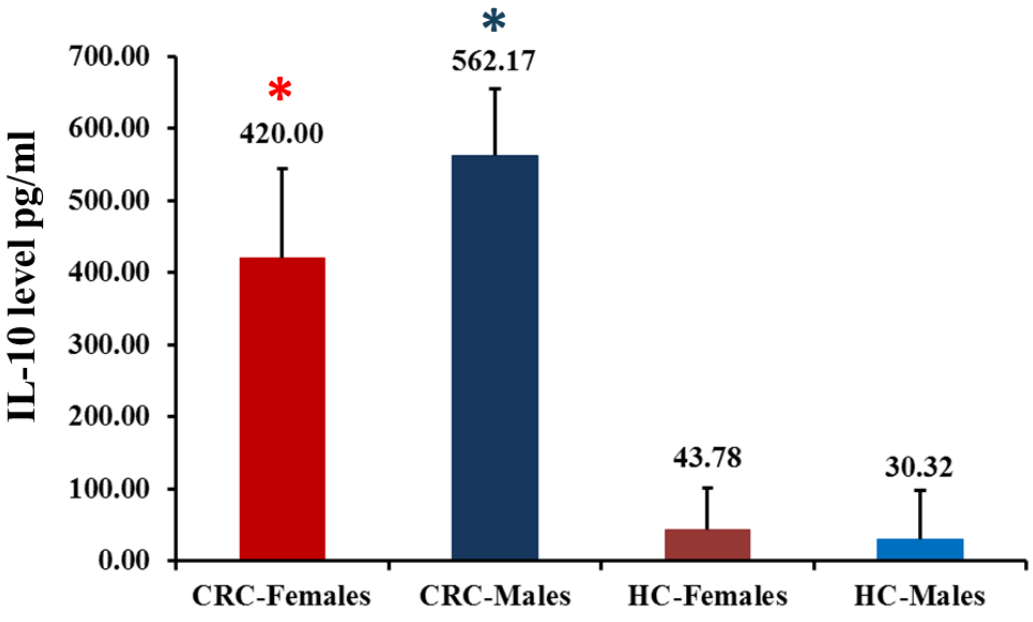

Figure 3. Serum level of IL-10 according to the gender of both CRC patients and H.C. groups.

1.4. Serum level of IL-10 according to age groups

The present findings reported a significant elevation in IL-10 level in the CRC group compared to H.C. according to age groups ($p<0.05$). A high level of IL-10 was detected in sera of the G4 group, representing 40-49 years, followed by G5 of age interval 50-59 years, Figure 4.

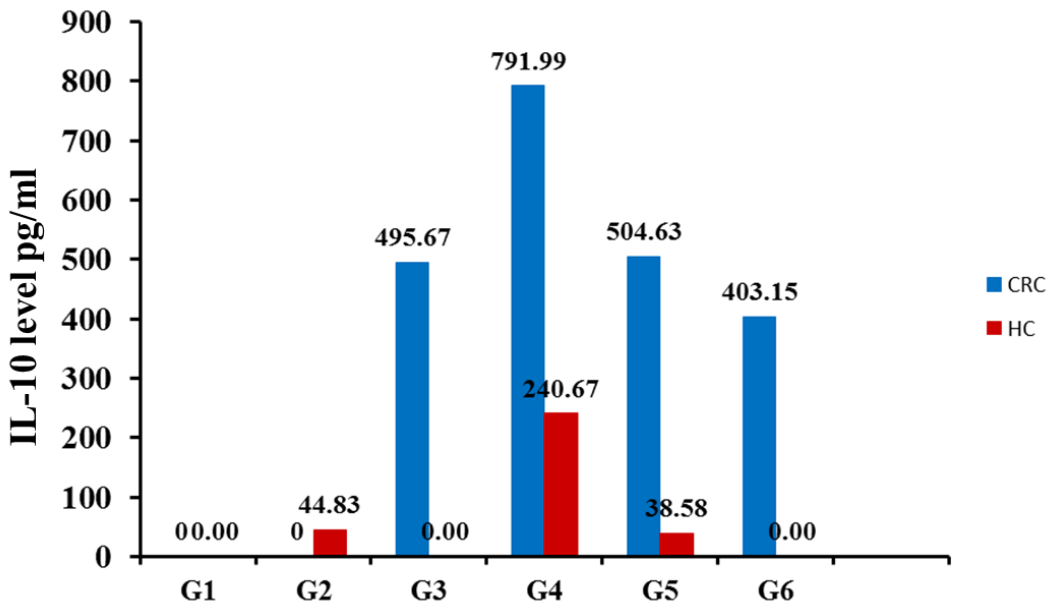

Figure 4. Serum level of IL-10 according to different age groups(G1-G6) of CRC patients and healthy control group.

1.5. Serum level of IL-10 according to Body Mass Index

The outcome of this study showed no significant changes in serum IL-10 level in the CRC group concerning their body mass index (BMI) ($p<0.05$), as shown in Figure5.

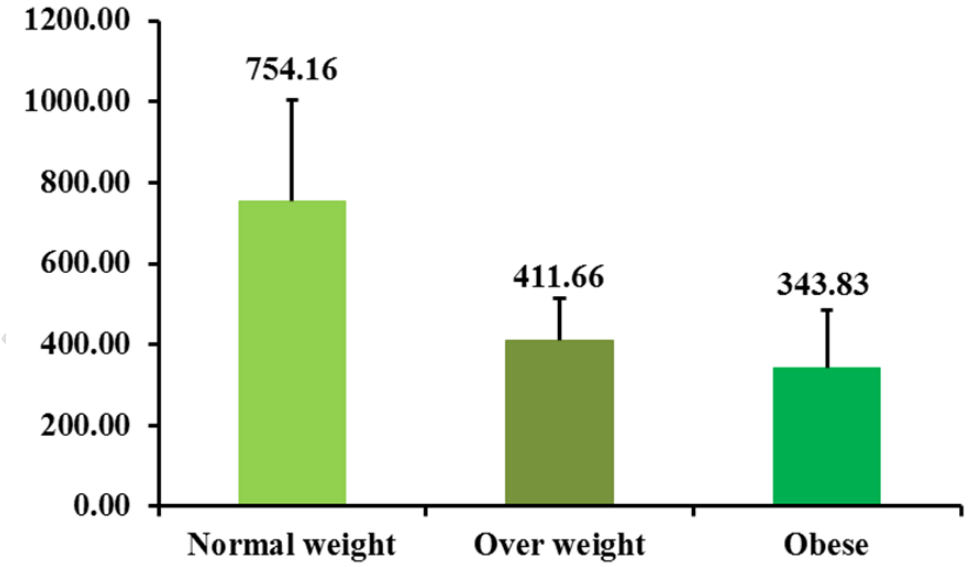

Figure 5. Serum level of IL-10 according to body mass index of the CRC patients.

1.6. Pearson's correlation between IL-10 level and some study subjects parameters

The correlation between IL-10 level and age, gender, and body mass index of the CRC group and the healthy controls was evaluated as shown in Figures 6,7&8. . Non-significant weak negative correlation was observed between IL-10 serum levels and ages of the CRC patients with Pearson's correlation = -0.166. In comparison, a non-significant weak positive correlation was detected between IL-10 serum levels and the control group's age with Pearson's correlation = 0.08. Moreover, a non-significant weak negative correlation between IL-10 and BMI in the CRC group was found with Pearson's correlation = -0.33.

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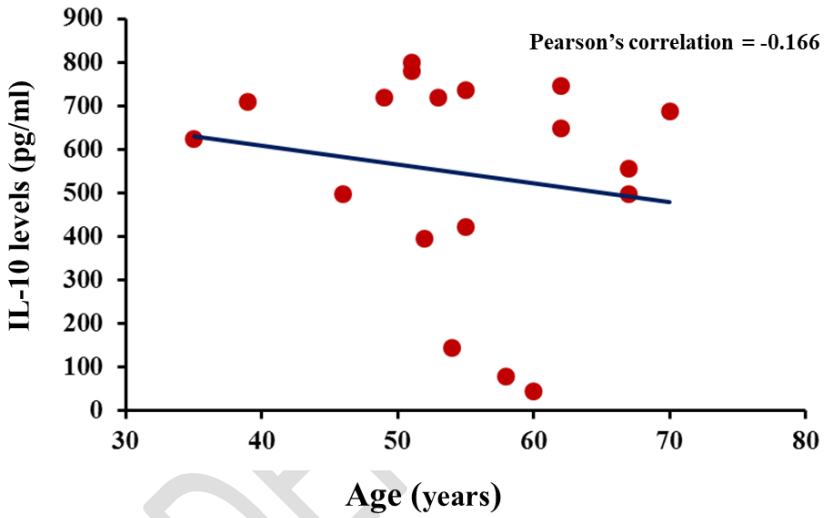

Figure6. A non-significant weak negative correlation between the IL-10 serum level and the ages of the CRC group.

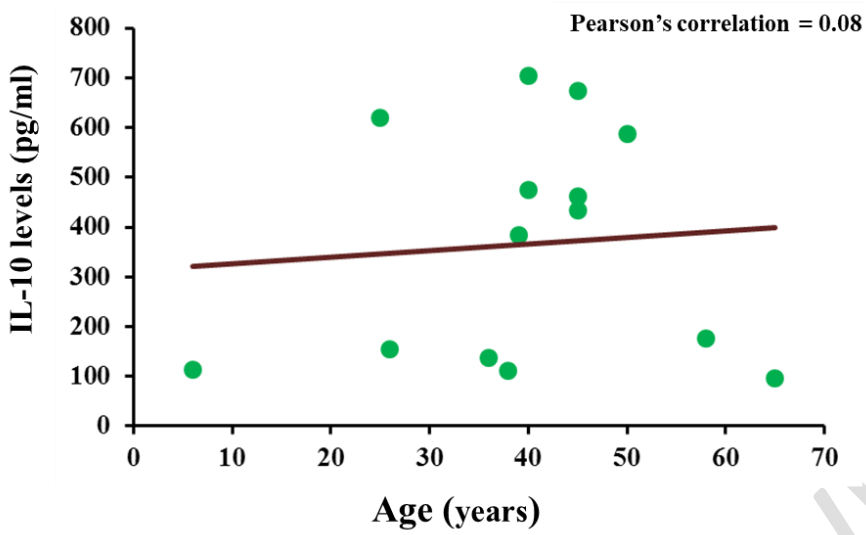

Figure7. A non-significant weak positive correlation between IL-10 levels and ages of the control group.

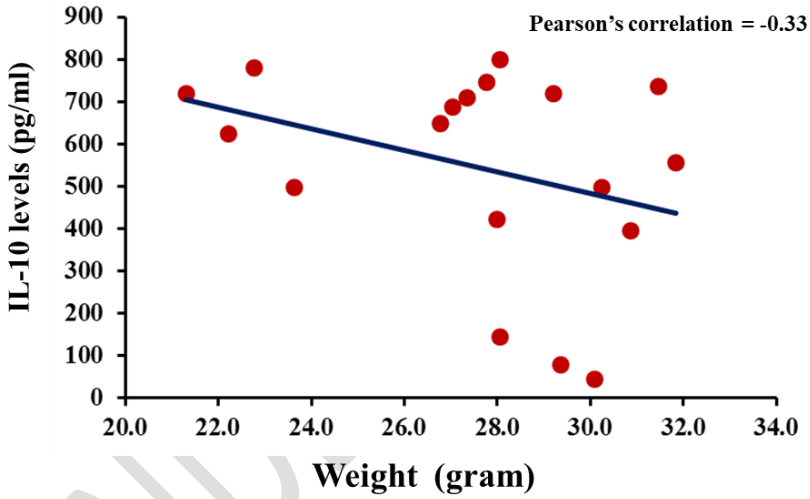

Figure8. A non-significant weak negative correlation Pearson's correlation between IL-10 and BMI in CRC group

Discussion

CRC is a common form of human cancer, and it has a severe influence on human health owing to its morbidity and mortality [14]. Recently, the CRC showed a significant increase in incidence rate after 2007 ($p < 0.001$) in Iraq. Females and males were equally infected [5]. The high CRC trend in Iraq was identical to other low- and middle-income countries, where the incidence was rising compared to high-income countries with steadying or decreasing percentages [2,15]

The present study results showed that the age group 50-59 years old was more affected. This finding is in line with [16], who found that CRC mainly affects persons aged 55 years. Also, it is worthwhile to mention the several cases that were recorded in the recent study within the 30-39-year-old. Which, may indicate that CRC has revealed an increased incidence among young individuals, as also observed previously by[17]. Furthermore, increased CRC incidence among individuals under age 50 was emphasized in the United States despite the decrease in CRC incidence and mortality, representing new alarming [18]. The CRC infection of young people may be attributed to the changes in food habits, sedentary lifestyles, family history, environmental pollution, and obesity, which are assumed as risk factors for CRCs [19,20]

A significantly high level of IL-10 was recorded in the sera of CRC patients compared to healthy controls in the present study. Similar findings were documented in CRC patients by [12] and concluded that elevated serum concentration of B7-H1 and IL-10 might have an important role in developing this type of cancer. The same was true with the results of [21], who pointed out that IL-10 and IL-18 are intensely expressed in CRC persons' sera and propose IL-10 and IL-18 as a beneficial indicator of the prognosis of CRC patients. Moreover, in a previous investigation, [22] proved that IL-10 sera concentration of colorectal and gastric cancer patients was increased significantly compared to the healthy control.

In recent, many studies have shown that circulating cytokine levels gave prognostic information in CRC patients. And these pro-inflammatory cytokines have a significant role in tumor growth and progression [23,24]. The massive release of these cytokines and the acute phase protein to the CRC circulating system is considered a hallmark for this cancer type [25]. Moreover, the present finding consistent with the results of another study that documented the significant elevation of IL-10 in the CRC patients' sera. They concluded that IL-10 might possess a role in tumor development. Also, the high level of it was strongly related to the

progression of CRC [26]. Moreover, Zhao et al. [27] reviewed twenty-one published articles to evaluate serum levels of IL-10 and the clinical manifestation of cancer patients. They concluded that high IL-10 concentration in sera of cancer patients predicts worse outcomes in them.

On the other hand, Abtahi et al.[28] demonstrated that IL-10 in CRC patients' sera was significantly less than the control, whereas it is raised in poor prognosis patients. They confirmed the dual role of serum IL-10 in the initiation and progression of CRC.

Interleukin-10 is a potent pro-inflammatory cytokine secreted by different cells, for instance, monocytes, macrophages, Th2, and Treg cells. It achieves its role by activating the STATI, STAT3, P13K, and p38 mitogen-activated protein Kinases mechanisms. Its essential function is to inhibit Th1 cytokines, the classically activated/M1 macrophage inflammatory gene expression, and antigen presentation [29]. Also, it is known as an essential anti-inflammatory and immunosuppressive cytokine. It inhibits the Th1 activation, prevents antigen-presenting cells from gaining entrance to tumor antigens and then presents the antigen to T-cells, and down-regulates surface expression of costimulatory molecules on the tumor [8,30]. Several researchers assumed that IL-10 might help tumor cells' escaping mechanisms from the immune surveillance and enhance tumor growth because of its immunosuppressive and anti-inflammatory characteristics. Alternatively, many studies showed that IL-10 may regulate angiogenesis in numerous cancers and is supposed to achieve a protective and preventive activity against tumors [31,32]. Also, there was no significant correlation between IL-10 level and age, gender, and the BMI of the CRC patients was recorded in the current study. Similar findings were reported in the recent investigation within CRC patients in China [12].

A limitation of this work is the relatively few numbers of patients involved due to the quarantine situation owing to pandemic COVID-19. As a result, we suggest that the present results be validated in further a larger group of patients to confirm the diagnostic and prognostic roles of detecting serum IL-10 in CRC patients.

Conclusion

In conclusion, Elevated serum levels of IL-10 of CRC patients compared to healthy control may have a profound action on the development and progression of CRC. Serum expression of IL-10 was not correlated with age or gender, or BMI and may play an essential role in the growth of

CRC. The high expression of IL-10 in CRC patients' sera, suggesting its usefulness for detecting CRC patients' prognosis.

Consent

The blood samples and information were collected from governmental hospitals under the approval of oncologist physicians and patients' acceptance.

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