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

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ABSTRACT

Colorectal cancer (CRC) is classified as one of the most prevalent cancer types worldwide, with high morbidity and mortality rates. Patients of CRC have been shown to express a detectable cytokine in serum which contributes to cancer pathogenesis. Therefore, the serum interleukin 10 (IL-10) level 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 confirmation of 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.
1. 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.

2. MATERIALS AND METHODS

2.1 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.

2.1.1 Sample’s selection and collection

Peripheral blood samples were taken from thirty-one post-diagnosed patients with late-stage 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.

2.2 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 xg. 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.

2.2.1 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 (Fig. 1).

### 2.3 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.

### 3. RESULTS

#### 3.1 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) Kg/ m², as shown in Tables 1, 2, and 3.

![Figure 1: ELISA standard curve of IL-10](image)

**Fig. 1.** 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.

| Groups             | Females (%) | Males (%) | Total |
|--------------------|-------------|-----------|-------|
| Colorectal cancer  | 14 (45.1)   | 17 (45.8) | 31    |
| Healthy controls   | 23 (57.5)   | 17 (42.5) | 40    |
| Total              | 37 (52.1)   | 34 (47.8) | 71    |

Table 1. Distribution of study subjects according to gender
3.1.1 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). IL-10 estimation revealed significantly raised sensitivity for distinguishing between CRC patients and healthy controls, as illustrated in Fig. 2.

3.1.2 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 Fig. 3.

3.1.3 Serum level of IL-10 according to age groups

According to age groups, the present findings reported a significant elevation in IL-10 level in the CRC group compared to H.C. (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, Fig. 4.

3.1.4 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 Fig. 5.

3.1.5 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 Figs 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.

4. 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 worth mentioning the several cases recorded in the recent study within the 30-39-year-old. This may indicate that CRC has revealed an increased incidence among young individuals, as observed previously [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
Table 2. 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    |

Table 3. Distribution of the CRC patients according to body mass index (Kg/m²)

| Groups            | Normal (%) | Overweight (%) | Obesity (%) | Total |
|-------------------|------------|----------------|-------------|-------|
| Colorectal cancer patients | 9 (29)     | 16 (51.6)      | 6 (19.3)    | 31    |

Fig. 2. 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 ELISA test results (P<0.05)
Fig. 3. Serum level of IL-10 according to the gender of both CRC patients and H.C. groups

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

Fig. 5. Serum level of IL-10 according to body mass index of the CRC patients
Fig. 6. A non-significant weak negative correlation between the IL-10 serum level and the ages of the CRC group

Fig. 7. A non-significant weak positive correlation between IL-10 levels and ages of the control group

Fig. 8. A non-significant weak negative correlation Pearson's correlation between IL-10 and BMI in CRC group
5. 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 confirms 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. The scientific and ethics committee of the Biology Department, Science Faculty, Soran University approved this work.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A.Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. C.A. Cancer J Clin. 2018;68:394-424. Available: https://doi.org/10.3322/caac.21492

2. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. Gut. 2017;66(4):683–691. DOI: 10.1136/gutjnl-2015-310912

3. De Santis CE, Lin CC, Mariotto AB, et al. Cancer treatment and survivorship statistics. C.A. Cancer J Clin. 2014;64(4):252–71. DOI: 10.3322/caac.21235

4. Kuipers EJ, Grady WM, Lieberman D, Seufferlein T, Sung JJ, Boelens PG, et al. Colorectal cancer. Nature reviews. Disease primers. 2015;1:15065. Available: https://doi.org/10.1038/nrdp.2015.65

5. Hussain AM, Lafta RK. Cancer Trends in Iraq 2000-2016. Oman Med J. 2021;36(1):e219. DOI: 10.5001/omj.2021.18 PMID: 33552559.
6. Shnawa HB, Al-Majmaie Sh M. Oncology research productivity of Iraqi authors: A bibliometric Analysis during 1955–2019. Medico-legal Update. 2021;21(1):174-182. DOI:https://doi.org/10.37506/mlu.v21i1.230

7. Hoechst B, Ormandy LA, Ballmaier M, et al. A new population of myeloid-derived suppressor cells in hepatocellular carcinoma patients induces CD4 (+) CD25(+) Foxp3(+) T cells. Gastroenterology. 2008;135:234–43.

8. Moore KW, de Vaal Malefyt R, Coffman RL, O’Garra A. Interleukin-10 and the Interleukin-10 receptor. Annual Review of Immunology. 2001;19(1):683-765. Available:https://doi.org/10.1146/annurev.immunol.19.1.683

9. Wang MH, Helzlsouer KJ, Smith MW, Hoffman-Bolton JA, Clipp SL, Grinberg V, De Marzo AM, Isaacs WB, Drake CG, Shugart YY, Platzt EA. Association of interleukin-10 and other immune response- and obesity-related genes with prostate cancer microenvironment using lentivectors encoding shRNA against IL-10. Cancer Causes Control. 2013;24(2):335-41. DOI: 10.1007/s10552-012-0119-3. Epub 2012 Dec 8. PMID: 23224326.

10. Rossowska J, Anger N, Szczygiel A, et al. Reprogramming the murine colon cancer microenvironment using lentivectors encoding shRNA against IL-10 as a component of a potent DC-based chemoimmunotherapy. J Exp Clin Cancer Res. 2018;37:126. Available: https://doi.org/10.1186/s13046-018-0799-y

11. Wang P, Li C, Ma X, Gai X. Clinical significance of the combined measurement of serum B7-H1 and interleukin-10 in colorectal cancer patients. Medicine (Baltimore). 2020;99(18):e20044. DOI: 10.1097/MD.0000000000020044

12. Liu CY, Xie WG, Wu S, Tian J-W, Li J. A comparative study on inflammatory factors and immune functions of lung cancer and pulmonary ground-glass attenuation. Eur Rev Med Pharmacol Sci. 2017;21:4098–103.

13. Liu CY, Xie WG, Wu S, Tian J-W, Li J. A comparative study on inflammatory factors and immune functions of lung cancer and pulmonary ground-glass attenuation. Eur Rev Med Pharmacol Sci. 2017;21:4098–103.

14. Ress AL, Perakis S, Pichler M. microRNAs and Colorectal Cancer. Adv Exp Med Biol. 2015;889:89-103. DOI: 10.1007/978-3-319-23730-5_6 PMID: 26658998.

15. Al-Lawati NA, Al-Bahrami BJ, Al-Raisi SS, Al-Lawati JA. Twenty-year trends of cancer incidence in Omanis, 1996–2015. Oman Med J. 2019;34(4):361-387.

16. Andreu García M, Marzo M, Mascort J, Quintero E, García-Alfonso P, López-Ibor C, Castells A, et al. Prevención del Cáncer de Colon en España. Prevención del cáncer colorectal [Prevention of colorectal cancer]. Gastroenterol Hepatol. 2009;32(3):137-9. Spanish. DOI: 10.1016/j.gastrohep.2008.12.001 PMID: 19232778.

17. Goldvasser H, Purim O, Kudel Y, Shephelowich D, Shochat T, Shemesh-Bar L, et al. Colorectal cancer in young patients: is it a distinct clinical entity? Int J Clin Oncol. 2016;21(4):684-695. DOI: 10.1007/s10147-015-0935-z. PMID: 26820719.

18. Loomans-Kropp HA, Umar A. Increasing incidence of colorectal cancer in young adults. J Cancer Epidemiol. 2019;11:9841295. DOI: 10.1155/2019/9841295. PMID: 31827515 PMCID: PMC6885269.

19. Rosato V, Bosetti C, Levi F, Polesel J, Zucchetta P, Negri E, La Vecchia C. Risk factors for young-onset colorectal cancer. Cancer Causes Control. 2013;24(2):335-41. DOI: 10.1007/s10552-012-0119-3. Epub 2012 Dec 8. PMID: 23224326.

20. Lo AC, Soliman AS, Khaled HM, Aboelyazid A, Greenson JK. Lifestyle, occupational, and reproductive factors and risk of colorectal cancer. Dis Colon Rectum. 2010;53(5):830-7. DOI: 10.1007/DCR.0b013e3181d320b1. PMID: 20389219; PMCID: PMC3223860.

21. Li, B., Wang, F., Ma, C., Hao, T., Geng, L., & Jiang, H. Predictive value of IL-18 and IL-10 in the prognosis of patients with colorectal cancer. Oncology Letters. 2019;18:713-719. Available:https://doi.org/10.3892/ol.2019.10338

22. Abdulla A R, Ali AH, Al-Rawaq K J, Ibraheem A N. 2012. IL-10 serum level
estimation in Iraqi colorectal and gastric cancer patients. J Fac Med Baghdad. 2012; 54, (2): 167-171.

23. Birgisson H, Tsimogiannis K, Freyhult E, Kamali-Moghaddam M. Plasma Protein Profiling Reveal Osteoprotegerin as a Marker of Prognostic Impact for Colorectal Cancer. Transl Oncol. 2018; 11(4):1034-1043. DOI: 10.1016/j.tranon.2018.05.012. PMID: 29982101; PMCID: PMC6037900.

24. Gunawardene A, Dennett E, Larsen P. Prognostic value of multiple cytokine analysis in colorectal cancer: a systematic review. J Gastrointest Oncol. 2019;10(1):134-143. DOI: 10.21037/jgo.2018.07.11. PMID: 30788169.

25. Tuomisto AE, Mäkinen MJ, Väyrynen JP. Systemic inflammation in colorectal cancer: Underlying factors, effects, and prognostic significance. World J Gastroenterol. 2019; 25 (31): 4383-4404. DOI:https://dx.doi.org/10.3748/wjg.v25.i31.4383

26. Stanilov N, Miteva L, Deliysky T, Jovchev J, Stanilova S. Advanced colorectal cancer is associated with enhanced IL-23 and IL-10 serum levels. Lab medicine. 2010; 41(3):159-163. Available:https://doi.org/10.1309/LM7T43AQZIUPOWZ

27. Zhao S, Wu D, Wu P, Wang Z, Huang J. Serum IL-10 predicts worse outcome in cancer patients: A meta-analysis. PLoS One. 2015;10(10):e0139598. DOI:10.1371/journal.pone.0139598. PMID: 26440936

28. Abtahi S, Davani F, Mojtahedi Z, Hosseini SV, Bananzadeh A, Ghaderi A. Dual association of serum interleukin-10 levels with colorectal cancer. J Can Res Ther. 2017;13:252-6. DOI: 10.4103/0973-1482.199448

29. Carson WF, Kunkel SL. Type I and II cytokine superfamilies in inflammatory responses. In: Cavaillon JM, Singer M, editors. Inflammation: From Molecular and Cellular Mechanisms to the Clinic. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA. 2017;587-618. DOI: 10.1002/9783527692156.ch24

30. Mittal SK, Roche PA. Suppression of antigen presentation by IL-10. Curr Opin Immunol. 2015; 34:22-7. DOI: 10.1016/j.coi.2014.12.009. Epub 2015 Jan 16. PMID: 25597442.

31. Chang CC, Liu CD, Pan SF, Huang WH, Peng CW, Hsue HJ. Targeting of interleukin-10 receptor by a potential human interleukin-10 peptide efficiently blocks interleukin-10 pathway-dependent cell proliferation. Ci Ji Yi Xue Za Zhi. 2020;32(3):245-253. DOI: 10.4103/tcmj.tcmj_237_19 PMID: 32955521.

32. Huang S, Ullrich SE, Bar-Eli M. Regulation of tumor growth and metastasis by interleukin-10: the melanoma experience. J Interferon Cytokine Res. 1999;19:697–703.