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Gene Expression Of IL10 And IL18 As A Biomarker In Colorectal Cancer Patients

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Abstract :

The present study use to detect the expression of IL10 ,IL18 mRNA gene by using Real- time PCR (Relative gene expression) in CRC patients and the results found that the level of IL10 ,IL18 decrease in CRC Patients when compared with control groups.

Keywords: coleractal cancer**,** IL10 ,IL18 mRNA gene **,** Real- time PCR

1.Introdection

Interleukins (ILs) can be divided into several families with more than 40 subfamily members. They can interact with a variety of cells that alter the immune system and act on a wide range of cancers. In the past several years, ILs have attracted substantial attention because of their distinct roles in CRC that provide a new and promising strategy for CRC. In general, ILs facilitate CRC by promoting tumorigenesis ,tumour growth (Hai Ping et al ., 2016).

Several cytokines that modulate the immunologic response have been implicated in the development of cancer .Interleukin-10 (IL-10) is a multifunctional cytokine involved in both innate and adaptive immune response, and a wealth of evidence supports its regulatory role in carcinogenesis and tumor growth.In addition, increased circulating IL10 has been shown in patients diagnosed with different malignancies, suchas hepatocellular carcinoma, autoimmune cancers, and leukemia (Namaziet al .,2018).

Studies have shown that two family members (IL-10 and IL-22) are closely related to CRC, suggesting that they are potential therapeutic prospects. IL-10 was first discovered to be secreted by Th2 cells in mice in 1989, and since it inhibited the synthesis of IL-2 and IFN-γ, it was primarily represented as secreted cytokine synthesis inhibitory factor (CSIF). First discovered as IL-10-related T cell-derived inducible factor (IL-TIF), it can be produced by most of the lymphocyte subsets, which mainly are innate lymphoid cells(ILC3),Th17 and Th22 (Ouyanget al.,2019).

The interleukin-1 (IL-1) family comprises 11 members, including seven pro-inflammatory cytokines and one anti-inflammatory cytokine . Evidences have demonstrated that they are key regulators of multiple physiological and pathological processes, including innate immune and inflammatory responses .Among the IL-1 family, interleukin-18 (IL-18) is one of the best characterized. The protein encoded by the IL-18 gene, is essential for the response to the pathogens and activation of host defense mechanisms (Scott et al .,2018).

Interleukin 18 was originally discovered as a factor that enhanced IFN-γ production from anti-CD3-stimulated Th1 cells, especially in the presence of IL-12. Upon stimulation with IL-12, T cells develop into IL-18 receptor (IL-18R) expressing Th1 cells, which increase IFN-γ production in response to IL-18 stimulation. Therefore, IL-12 is a commitment factor that induces the development of Th1 cells. In contrast, IL-18 is a pro-inflammatory cytokine that facilitates type 1 responses .IL-18 is a cytokine that stimulates various cell types and has pleiotropic functions (Koubunet al ., 2019)

Although IL-18 also acts on non-polarized T cells, NK cells, NKT cells, B cells, DC and macrophages to produce IFN-γ in the presence of IL-12. Moreover, IL-18 without IL-12 but with IL-2 induces Th2 cytokine production from $CD4^+$ NKT cells,NK cells, and even established Th1 cells. Furthermore, IL-18 with IL-3 induces mast cells and basophils to produce IL-4 and IL-13. IL-18 stimulates both innate immunity and acquired immunity (Nakanishi et al., 2018).

Interleukin-18 belongs is an essential pro-inflammatory and immune regulatory cytokine . In clinical analyses, mRNA and protein expressions of IL-18 were decreased in tissues of colon cancer patients. This decreased expression of IL-18 was significantly correlated with the tumor size . Patients with IL-18-positive tumors had a better survival rate than patients with IL-18-negative tumors. Moreover, up-regulation of IL-18 inhibited colon cancer cell proliferation. The decreased expression of IL-18 in colon cancer was associated with prognosis and tumor proliferation. IL-18 may be considered a novel tumor suppressor and a potential therapeutic target for colon cancer patients. (Xiaodonget al ., 2020).

2. Material and method

2.A. Patients :

A case-control study was conducted in Imam Hussein medical city, Al-Kafeel Specialist Hospital and Imam Zain Al-Abidin Hospital in Holy Karbala between February 2020 to the March 2021. Individuals visited these centers with a suspension of colorectal cancer were undergone physical examinations, standardized colonoscopic examinations and histopathological examination.

Total of 40 Patients with confirmed CRC by two consensus histopathological reports were recruited in the studyincludes (Male 22 and female 18) age range (30-80) years.

Two ml of blood were obtained from all patients after they were confirmed to have colorectal cancer and when they visited the oncology center in Imam Hussein Medical City by puncture of the vein and placing it in Ethylenediaminetetraaceticacid (EDTA) tubes. RNA was extracted for gene expression study.

2.B. Control group

Twentyindividuals with the clinical diagnosis of hemorrhoid who visited the gastrointestinal center in Imam Hussein medical city, and Al-Kafeel Specialist Hospital in Holy Karbala were agreed to participate in our study. Colonoscopy were done tothose individuals as a diagnostic workup and their finding revealed negative endoscopy apart from bleeding hemorrhoid.Blood samples were obtained from all patients RNA extraction from these samples were done in order to be used later in the study.

3. Gene expression of Interleukin 10(IL10) by using real- time PCR

A total from (40) blood patients samples ,RNA was extracted to study the gene expression of IL-10 by using real-time PCR (Relative gene expression) (2-ddcT) methods in this method the level of expression of IL-10 gene in test samples as well as in control samples normalize with house - keeping for test samples ,as shown in figure (1) .

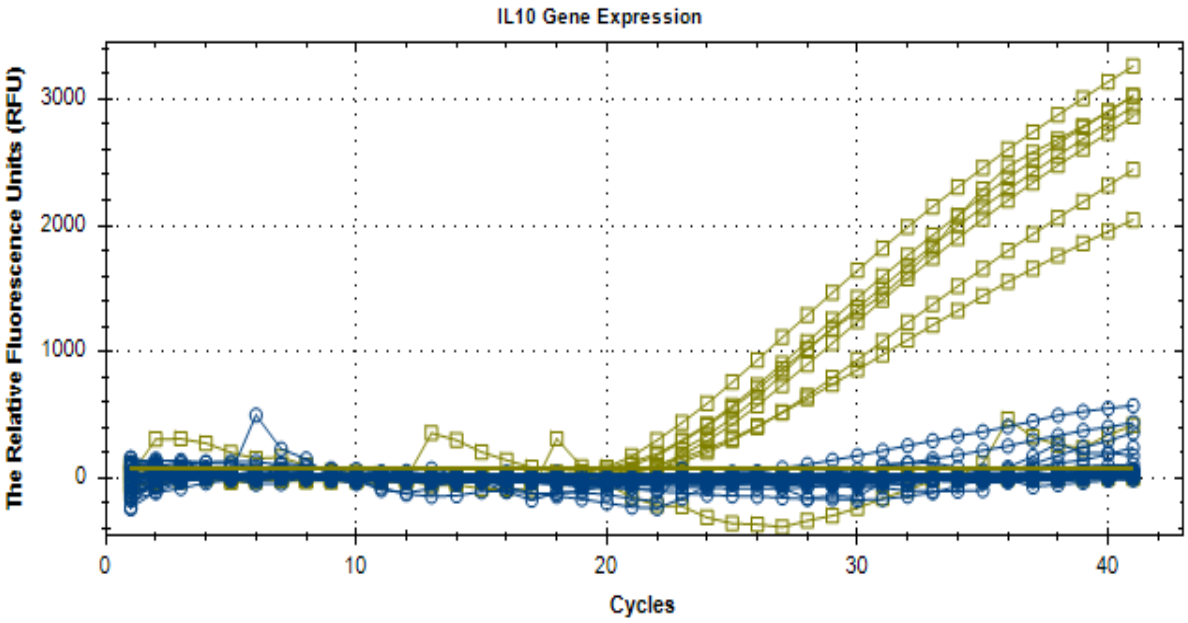

Figure (1): IL10 Gene expression level. This is the first run for 10 samples (Reference GAPDH) and 21, 13 samples (Control+ CRC Patients), represents amplification of Reference gene (GAPDH), represents amplification of samples (Control + CRC Patients).

The present study found that the expression of IL-10 gene very low in theCRC Patients when compared with control group. So expression of the gene is decrease in comparison with control set which increase more than 1.5 fold in compared with tumor tissues as shown in figure(2) and table (1).

Table (1): IL 10 Fold Gene Expression among Control and CRC Patients versus the reference gene (GAPDH).

Groups	N	Expression levels (2^-(ΔΔCt))	
		Mean	SD
Control	13	1.7	1.59
Patient	33	0.37	0.60
P value	< 0.0001*		

* represent a significant difference at p≤ 0.05.

The result in this study show that the value of level of IL-10 in CRC patients less than 1 , so expression is decrease of the gene in comparison with control set as shown in figure (2).

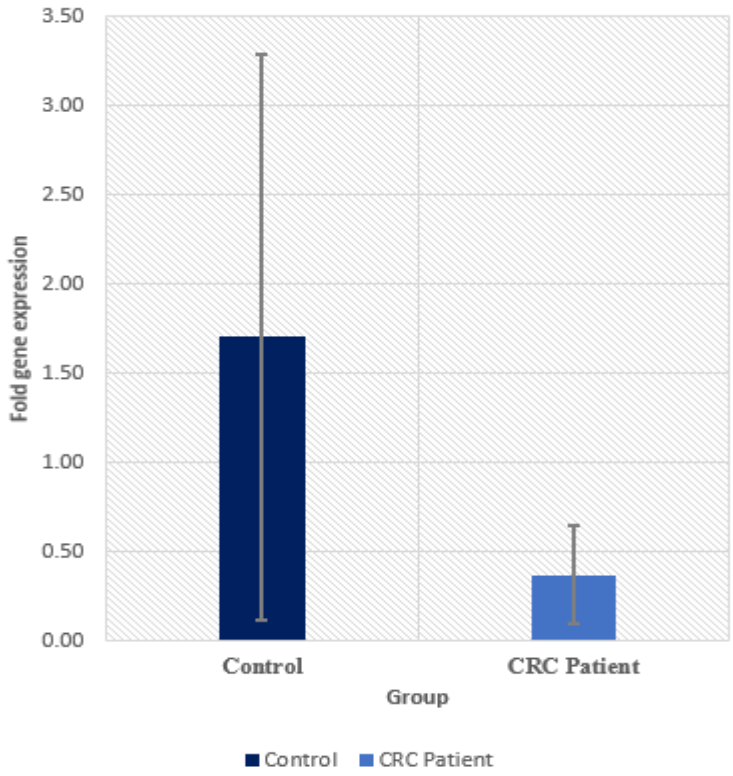

Figure (2): IL 10 Fold Gene Expression among Control and CRC Patients versus the reference gene (GAPDH)s

IL - 10 conducts to the recruitment and activation of Janus kinase (JAK) to initiate the down stream signaling pathway and transcription factors signal transducer and activator of transcription STAT, STAT3 ,STAT1 and STAT5 ,STAT3 play a dominant role in IL-10 signaling (Jingjinget al ., 2020).

IL-10 is crucial immunosuppression agent ,and the lack of IL -10 R in colorectal tissue could cause sever spontaneous colitis ,which poses a risk for CRC initiation (Shouvalet al ., 2014).

(BaosongLi et al ., 2019) found that the expression level of IL-10 was found lower in patient after CRC surgery than before ,and patient with recurrence CRC after surgery had significantly higher level of IL-10 ,this indicating that IL-10 can be a prognostic biomarker in CRC .

IL-10 expression is regulated at the transcriptional and post-transcriptional levels ,IL-10 appears to have considerable importance in the development of human cancer and it immune escape so it serve as a biomarker for prognostic diseases and for treatment (Mariaet al ., 2020).

IL-10 is encoded by the IL-10 gene which located on chromosome 1 and comprises 5 exons . Expression of IL-10 extensively regulated at the post-transcriptional level which my involved control of mRNA stability and by MicroRNA such as MiR -106 . (Schulte et al ., 2011).

The results of the present study consonant with (Shabnamet al ., 2017) which indicated the (IL-10) is considered an immune modulator cytokine, showing both antitumor and pro-tumor characteristics. Its role in the pathogenesis and progression of colorectal cancer depends on microenvironmental ,Mean serum IL-10 levels were significantly lower in CRC patients than in controls (P = 0.04). CRC patients with worse prognosis at the time of diagnosis tend to have higher levels of circulating IL-10 than those with better prognosis (P = 0.008). Receiver operating characteristics curve analysis demonstrated that IL-10 levels in the sera of CRC patients can be used as a prognostic biomarker in CRC patients .Therefore, lower IL-10 levels were associated with higher risk of the disease, its higher levels were associated with a poorer prognosis .

The results of the present study was disagreed with (Seung-Yong et al .,2021) which indicated IL-10 is produced not only by immune cells but also by cancer cells themselves. Many studies have examined the ability of IL-10 to suppress antitumor immunity. For example, IL-10 secreted by peritoneal monocytes down regulates cytokine production and T-cell proliferation in ovarian cancers. Patients with more advanced CRC have higher serum IL-10 levels and serum IL-10 has been shown to affect the prognosis of colon cancer patients.

(Andrzejet al ., 2009)found the serum cytokine levels in patients with CRC characterized by the stimulated production of monocyte / macrophage pro-inflammatory cytokines in the presence of normal circulating levels of IL-10. The level of IL-10 was not significantly different from the normal value. The CRC patients had a significantly lower level of IL-10 than the Ulcerative colitis (UC) patients. The cytokine levels in the CRC patients showed no significant differences (0.6255).

The results of the present study was disagreed with (Braham et al ., 2017) which indicated the Interleukin 10 is considered an immune modulator cytokine, showing both antitumor and pro-tumor characteristics. Its role in the pathogenesis and progression of colorectal cancer depends on microenvironmental milieu mean serum IL-10 levels were significantly higher in CRC patients than in controls. CRC patients with worse prognosis at the time of diagnosis tend to have higher levels of circulating IL-10 than those with better prognosis.

4.Gene expression of Interleukin18(IL18) by using real- timePCR :

A total from (40) blood patients samples and 20 blood control samples ,RNA was extracted to study the gene expression of IL18 by using real- time PCR (Relative gene expression) (2-ddcT) technique in this method the level of expression of IL-18 gene in test samples as well as in control samples normalize with house - keeping gene for test samples ,as shown in figure (3) .

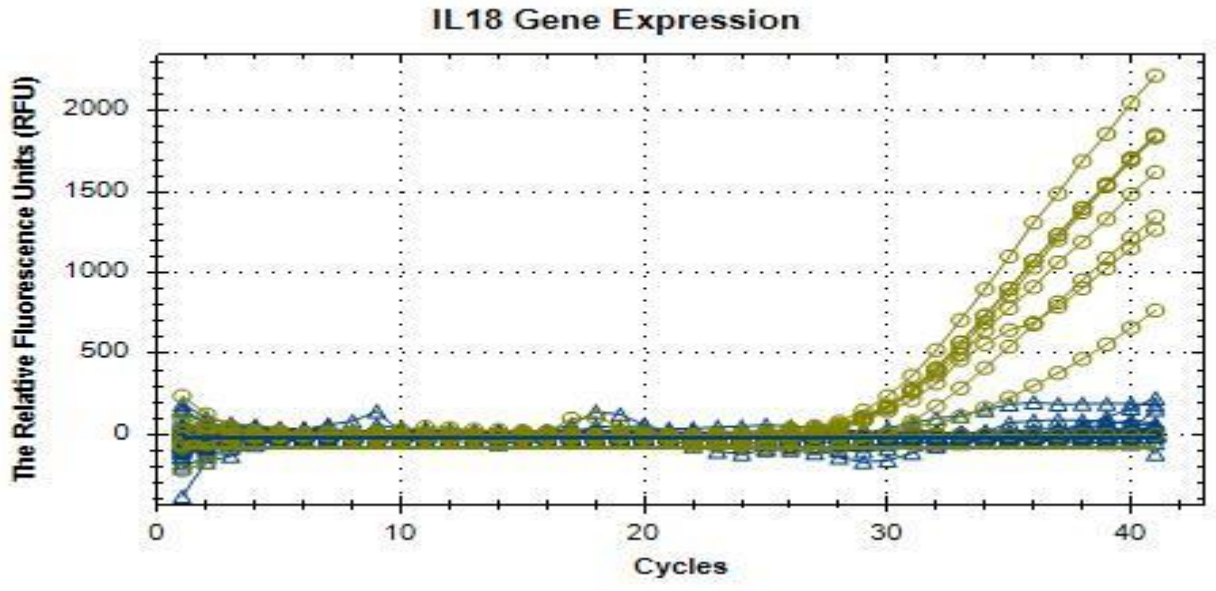

Figure (3): IL18 Gene expression level. This is the first run for 10 samples (Reference GAPDH) and 5 samples (Control+CRC Patients), represents amplification of Reference gene (GAPDH), represents amplification of samples (Control + CRC Patients).

The results found that the expression of IL18 mRNA gene were very low in theCRC Patients when compared with control group as shown in Table (2) .

Table (2): IL 18 Fold Gene Expression among Control and CRC Patients versus the reference gene (GAPDH).

Groups	N	Expression levels (2^-(ΔΔCt))	
		Mean	SD

Control	**13**	**3.05**	**4.79**
Patient	**33**	**0.22**	**0.05**
P value	**< 0.0001***		

* represent a significant difference at p≤ 0.05

The result in this study show that the value of level of fold gene expression of IL18 in CRC patients less than 1 , so expression of the gene is decrease in comparison with control set which increase more than 2fold in compared with tumor tissues as shown in figure (4).

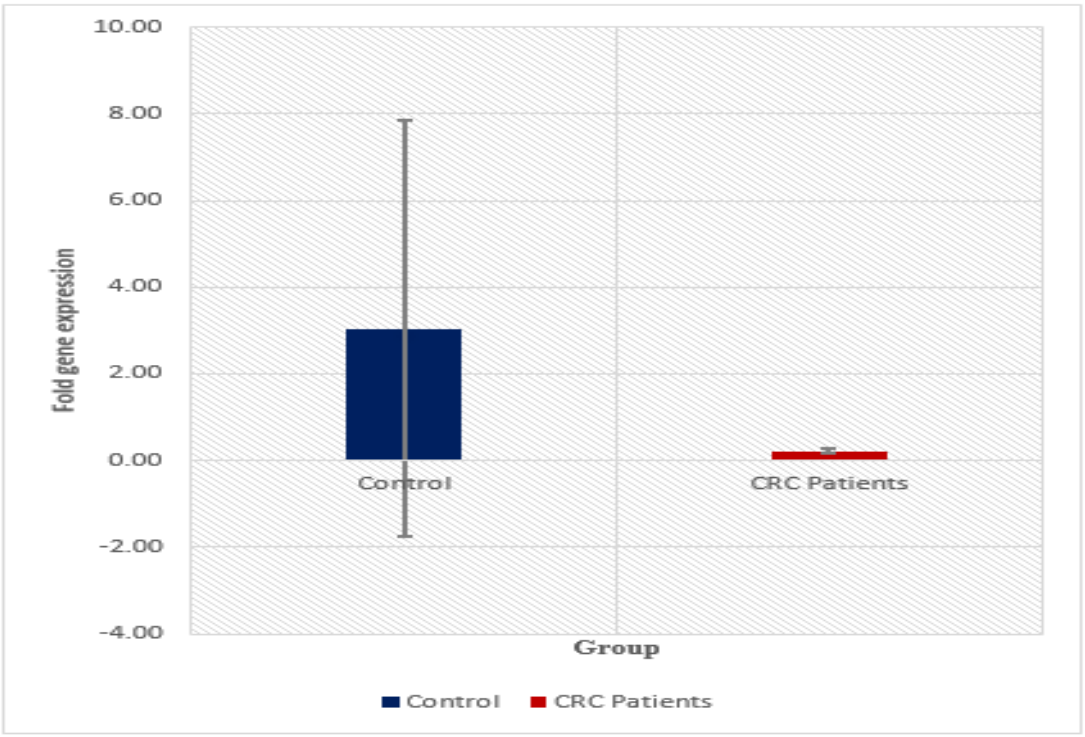

Figure (4): IL -18 Fold Gene Expression among Control and CRC Patients versus the reference gene (GAPDH)

Among the IL-1 family, interleukin18 (IL-18) is one of the best characterized. The protein encoded by the IL-18 gene, located at 11q23.1, is essential for the response to the pathogens and activation of host defense mechanisms. IL-18 has been shown to be a mediate product of activation by NOD-like receptor pyrin domain-containing protein 3 (NLRP3) inflammasome/caspase-1 signaling pathway. It is secreted mainly by macrophages and dendritic cells and stimulates interferon-γ (IFN-γ) production by natural killer (NK) cells and thymus-dependent lymphocyte(T cells) (Koubun Yasuda et al ., 2019).

Interleukin-18 (IL18, also known as interferon-gamma inducing factor) isa protein which in humans encoded by the IL18 gene. The protein encoded by this gene is a proinflammatory cytokine., IL-18 can modulate both innate

and adaptive immunity and its dysregulation can cause autoimmune or inflammatory diseases. Recently found , aberrant low expression of IL-18 has been identified in some digestive system cancers, such as esophageal cancer and oral squamous cellcarcinoma(Li et al .,2018).

(XiaodongFenget al ., 2020) found that IL-18 was significantly lower in colon cancer tissues than normal tissues, and this down regulated expression was happened on the early stage of the disease, The data revealed that low IL-18 expression in colon cancer tissues was associated with tumor size and AJCC stage and implicated that IL-18 is a prognostic factor foroverall survival (OS). Furthermore, the results indicate that IL-18 markedly represses colon cancer cell proliferation.The results of current study disagree with (NasibehHosseiniet al .,2019) who indicated that the cytokine level was increased significantly in cancer patients .

(Baosong Li et al ., 2019) have demonstrated that the expression levels of IL-18 in the serum of patients with CRC were statistically higher than those of the control group. And the expressions of IL-18 of patients with reoccurred CRC after the operation were significantly higher than that of patients without recurrence of colorectal cancer in the study group. The expressions of IL-10 and IL-18 in CRC patients were not statistically related with factors including age, gender and body mass index ,but were in statistical relation to factors such as the Dukes staging, tumor size, histological grades and the distant metastasis of cancer cells a gradual decrease of the expression of IL-10 and IL-18 in colorectal cancer patients surfaced after surgery and thus the expression levels of IL-10 and IL-18 in the serum 7 days after the operation were statistically lower than those before the operation ($P<0.05$).

(Jablonska et al .,2005) found that the expression of IL-18 in the serum of patients with oral squamous cell carcinoma 3 weeks after the surgery was lower than that before surgery, and the study by (Becker et al .,2005) revealed that patients with colon cancer had a lower expression of serum IL-10 after the treatment compared with that before thetreatment.

Conclusions:

1-The expression of IL-10 and IL-18gene low in theCRC Patients when compared with control group.

2- IL-18 and IL-10 arepotential therapeutic target for colon cancer patients .

3-The decreased expression of IL-18 in colon cancer was associated with prognosis and tumor proliferation.

4-Several cytokines such as Interleukin-10that modulate the immunologic response have been implicated in the development of cancer .

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