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Estimation of immunological markers (IL1Alpha, IL-6 and CRP) in colorectal cancer in Iraqi patients

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Abstract

Cytokines are A type of protein that is made by certain immune and non-immune cells and has an effect on the immune system. Some cytokines stimulate the immune system and others slow it down. 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, angiogenesis, and cancer cell invasion and metastasis and inhibit CRC via complex pathways. The Bioassay Technology Human Interleukin 6, Interleukin 1 alpha (E0090Hu) and C-reactive protein Kits was used. The quantitative determination of human interleukins concentrations and CRP were used in serum, plasma, cell culture supernatant, cell lysates and tissue homogenates .One hundred and twenty blood samples were collected from patients in gastrointestinal and hepatic hospital during the period from Feb 2019 to Feb 2020. Results revealed that the measuring the interleukin-alpha and intravenous blood levels of the subjects in our sample were different, but the difference with the other groups was very small, where the concentration rate was reported in the 699.84 ±51.39 colorectal cancer group and was similar to the other three groups, the average concentration of interleukin 6 in samples of patients with colorectal cancer was 48.81 + - 15.96 compared to the remaining groups, The analyses of the serum CRP concentration measurement were in patients with colorectal cancer, the concentration ratio was 31.45 ±7.94 relative to the performance of the other groups.

Keywords : Cytokines, Interleukin, CRC, CRP, Serum, Plasma.

Introduction

Cytokines are tumor necrosis factor (TNF) and interleukin-6 (IL-6) are typically observed as central players in CRC, motivating stimulation of the key oncogenic transcription factors nuclear factor-κB (NF-κB) and indicator transducer and activator of transcription 3 (STAT3), correspondingly, in intestinal epithelial cells to support proliferation and fight to apoptosis (Grivennikov et al., 2010). Further newly, cytokines through similar biochemical functions — including IL-11, IL-17A and IL-22 — have increased helpfulness as architects of both human and mouse CRC, other cytokines such as interferon-γ (IFNγ), IL-15 and IL-18 — stimulate defensive host immunity mediated by cytotoxic cell types, particularly CD8+ cytotoxic T lymphocytes (CTLs). Regulatory T (TReg) cells are necessary for the control of intestinal infection, owed in part to their making of the anti-inflammatory cytokines IL-10 and altering growth factor-β (TGFβ). While this oppressive role of TReg cells may be harmful due to the damage of protective immune reactions, it may also be advantageous for the limitation of pro-tumorigenic inflammation (Whiteside, 2012) . In spite of an imposing volume of associate sign from mouse disease models and thorough analyses of human tissue, variation of the CRC cytokine environment has infrequently been endeavored in the clinic.

IL-1β, an effective activator of NF-κB, is stated at high points in several cancer types and expression rises during development of CRC (Voronov & Apte, 2002; Cui et al.,2012). IL-1β can activate the WNT-β-catenin path in CRC by deactivating GSK3β (Kaler et al.,2009), and it makes mesenchymal and stemless structures, counting improved colony-forming capacity, appearance of stem ness genes such as *BMI1* and *NES* (which encodes nestin), and improved

fighting to chemotherapy (Li et al.,2012). Zinc portion E-box-binding homeobox 1 (ZEB1), an imperative pro-mesenchymal transcription aspect, has a critical role in these routes (Li et al.,2012). In the DSS–AOM model, neutrophils transport large amounts of IL-1β to the tumor micro environment. Obstruction of IL-1β using soluble IL-1 receptor antagonist diminishes tumor penetration by inflammatory cells, countenance of IL-6 by mononuclear cells and tumor formation (Wang et al., 2014). High levels of IL-1β are apparent in cancers from non-colitic *Apc*Δ468 mice. Stimulatingly, this does not arise in the lack of nuclear receptor RORγt (Blatner et al., 2012).

IL-6 is formed by different cell types and is a crucial mediator of inflammation and immunity (Taniguchi & Karin, 2014) . The IL-6 receptor transduces signs via gp130 (also known as IL-6Rβ), which is the communal receptor chain of the IL-6 family, and is a resilient inducer of STAT3 activation. IL-6 has some important roles in tumor progression, driving processes such as propagation, immigration and angiogenesis(Bollrath et al., 2009). Mouse and human studies have stressed its role in both CAC and sporadic CRC. IL-6-dependent STAT3 signaling is a serious promoter of cancer cell proliferation and persistence in the DSS–AOM model (Grivennikov et al., 2009 ; Gao et al., 2013). IL-6-prompted STAT3 signaling in myeloid cells was lately designated as a tumor relapse mechanism following radiotherapy (Lin et al.,2013). Especially, this consequence was speciously mediated by Toll-like receptor 9 (TLR9) signaling in myeloid cells that were conscripted to degenerating tumours (Dejea et al., 2014). IL-6 is formed by cancer-related mesenchymal stem cells and has pro-tumorigenic effects in human CRC through the stimulation of Notch 1 and CD44 expression (Maekawa et al., 2013). A novel potential driver of IL-6 production in human CRC was newly predictable in the form of bacterial biofilms on the colonic mucosa of patients with CRC (Rohini et al., 2008).

Material and Methods

Collection of blood samples: Blood samples were collected from patients with both malignant and benign tumors. Blood samples were collected from patients with colon and rectal ulcers and a group of healthy people, then put in a gel tube, segregated blood samples, and separated serum was taken and placed in an Eppendorf tube. In the analysis, was adopted for used to estimate the ratio of interleukins and C-reactive protein for all permitted samples.

Immunological Test by Elisa technique:

Human Interleukin 6 (IL-6): The Bioassay Technology Human Interleukin 6 kits (E0090Hu) was used. The quantitative determination of human interleukin 6 concentration was used in serum, plasma, cell culture supernatant, cell lysates and tissue homogenates.

Principle: This assay employs the quantitative sandwich enzyme immunoassay technique. After removing any unbound substance, a biotin –conjugated antibody specific for IL-6 was added to the wells .After washing, avidin conjugated Horseradish peroxidase (HRP) was added to the wells. After washing, a substrate solution was added to the wells and color develop in proportion to the amount of IL-6 bound in the initial step. The color development was stopped and intensity of color was measured.

Reagent using in kits :

Table (1) : Reagent using in kit

Components	Quantity
ELISA plate	12x8 coated microwells (96well)
Standard solution	2vial (0.5ml x1)
Standard Diluent	3ml x1
Biotin Antibody	120µl
Biotin Antibody Diluent	15 ml
HRP- Avidin	120 µl
HRP-Avidin diluents	15 ml
Sample Diluent	50 ml
Wash buffer	20 ml
TMB- Substrate	10 ml
Stop solution	10 ml

Reagent preparation Biotin –Antibody: Before opening, the vial was centrifuged, and 100- fold dilution was suggested (10µl of biotin antibody with 990 µl of biotin –antibody diluents).

HRP- Avidin: Before opening, the vial was centrifuged, and 100- fold dilution was suggested (10 µl of HRP- avidin was mixed with 990 µl of HRP-avidin diluents).

Wash buffer: The vial was warmed up to room temperature if crystals have formed in the concentrate ,and mixed gently until the crystal have completely dissolved ,500ml of the wash buffer was prepared by dilute 20ml of wash buffer concentrate into deionized or distilled water.

Standard: Before use ,(IL-6) Standard vial was centrifuged at 6000-10000 rpm for 30 s. The standards have the following concentration IL-6.

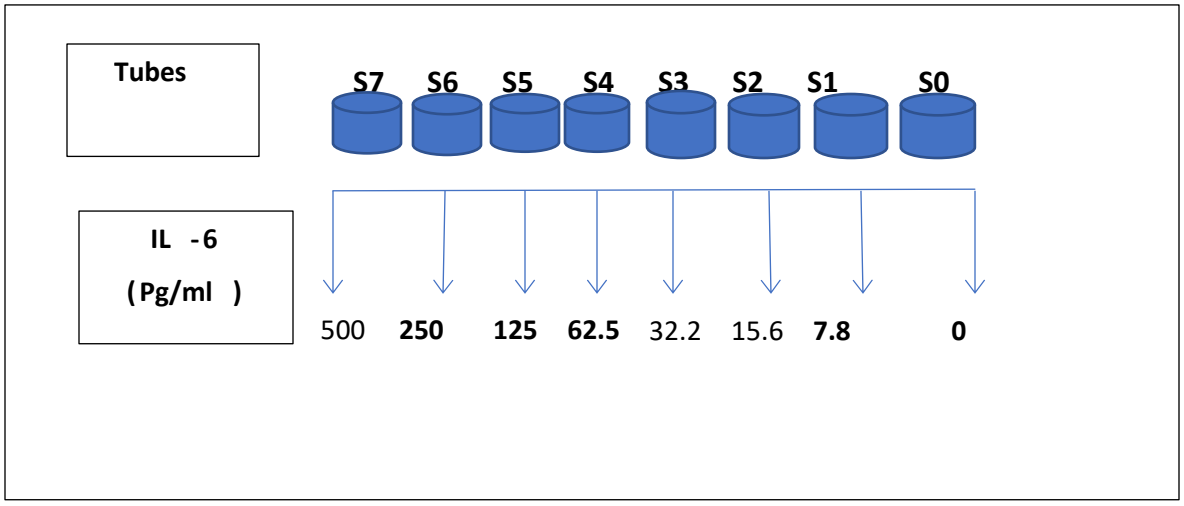

Figure (1): Standard Preparation

Assay procedure: The procedure was summarized in the following :

1. Reagent, samples and standards were prepared as instructed .
2. One hundred µL standards or samples were added to each well and incubated 2hrat 37ºC.

3.The liquid of each well was removed and did not wash.

4. One hundred µl of biotin antibody (1x) was added to each well and incubated 1hr at 37º C then aspirated and washed 3 times.
5. One hundred µL of HRP –avidin (1x) was added to each well and incubated 1hr at 37ºC then aspirated and wash 3 times.
6. Ninety µof TMB substrate was added to each well and incubated 15-30 min at 37ºC, protected from light.
7. Fifty µL of stop solution was added to each well and read at 450 nm within 5 minutes.

IL-Alpha 1: The same steps were used to measure the quantity of interleukin-6, except that the difference was only between interleukin-1 alpha and the same steps.

Determination of C- reactive protein principle: Serum C- reactive protein (CRP) at 6 mg/ L or higher causes a visible agglutination on slide of a suspension of latex particles coated with anti-human C- reactive protein.

Reagents: Suspension of latex particles coated with anti-human Creactive protein , sodium azide 0.95 g/L , borate buffer 100 mmol/L ,PH8.2. C- : Negative Control ; Serum containing CRP < 6 mg / L . C+ : Positive Control : Human serum containing CRP > 6 mg /L .

Procedure:

1.Test reagents and samples were brought to room temperature (18- 25 ºC).

2.The 50 µL of sample and 1 drop of each (negative & Positive) control were placed into separate circle on the test card.

3.The latex vial was mixed gently and repeatedly until complete resuspension of the latex particles .one drop of reagent (A) was added to each circle next to sample to be tested, and mixed together.

4. Cards were rotated at 100 rpm for 2 minutes.

Reading: Positive results: The presences of visible agglutination were examined within a minute after removing the card from the rotator.

Negative results: The absence of a visible agglutination indicated a negative reaction for CRP.

Statistical Analysis: To detect the effect of different factors on study parameters, the Statistical Analysis Method- SAS (2012) software was used. The LSD test (Analysis of Variation-ANOVA) was used for important comparison of means. The Chi-square test was used to make a significant percentage comparison (0.05 and 0.01 probability). Estimation of the coefficient of correlation between variables in this analysis.

Results and Discussion

Immunological Marker related to CRC :

Interleukin – Alpha 1:

The results of measuring the interleukin-alpha and intravenous blood levels of the subjects in our sample were different, but the difference with the other groups was very small, where the concentration rate was reported in the 699.84 ±51.39 colorectal cancer group and was similar to

the other three groups, and there was no substantial difference (Table 2).

Table(2): Comparison between difference groups in IL-1beta and IL-6

Group	Mean ± SE	
	IL-1beta	IL-6
Cancer	699.84 ± 51.39 a	48.81 ± 15.96 a
Ulcerative	810.94 ± 153.33 a	22.64 ± 12.69 ab
Polyp	550.67 ± 51.67 a	7.56 ± 1.85 b
Control	757.00 ± 220.74 a	24.31 ± 16.88 ab
LSD value	421.20 NS	24.20 *
P-value	0.747	0.0484

Means having with the different letters in same column differed significantly . * (P≤0.05.

Interleukin 6: The findings of the blood concentration calculation of interleukin 6 in patients for the four groups included in the analysis showed that they varied, as the average concentration of interleukin 6 in samples of patients with colorectal cancer was 48.81 ± 15.96 compared to the remaining groups. There is a significant difference (P≤0.05), as the highest percentage rate has been registered. Then there is the group of stable individuals in the CRC group, then a group of colon and rectal ulcers, then the polyps (Table 2).

C-reactive Protein: The analyses of the serum CRP concentration measurement were very different from those of the remaining marquees. In patients with colorectal cancer, the concentration ratio was 31.45 ±7.94 relative to the performance of the other groups, which was significantly different (P≤ 0.05) and improved from the majority of the other groups (Table 3).

Table (3): Mean of C-reactive Protein between difference groups.

Group	Mean ± SE
	CRP
Cancer	31.45 ± 7.94 a
Ulcerative	7.88 ± 2.01 b
Polyp	5.52 ± 1.72 b
Control	8.60 ± 3.13 b
LSD value	22.509 *
P-value	0.0359

Means having with the different letters in same column differed significantly . * (P≤0.05).

A dual-function cytokine present in the nucleus is interleukin 1 alpha. Nucleic acid binds to it. DNA also binds through receptors to the cell membrane and begins to establish signal transcription. According to the data of our research, the mean concentrations of interleukin 1 alpha in the blood of all samples are within the standard range, and no studies have shown an explanation of the two spheres or a rise in the concentration of this cytokine in colorectal cancer and other group samples in comparison to previous studies. On the other side, a group of researchers have conducted a study on the relationship between interleukin 1 alpha by testing its blood levels in patients with systemic sclerosis, as the study has shown that there is no significant difference between patients' serum and healthy individuals (Kaminska et al., 2000).

According to the results that appeared to us in measuring the levels of interleukin 6 in the blood serum, its concentration increased in the serum of patients with CRC cancer as well as in the tissues of colic ulcers and a small increase in samples of healthy people. According to studies conducted in the context of our results, where (Caro-Paton, 2005) in CRC samples reached elevated levels of IL-6, especially in the advanced stages of injury development, (Piancatelli et al., 1998) recorded this as well.

The following possibilities were used to explain this increase, and CEA has been shown to contribute to the release of several cytokines into the liver by Kupffer cells, including IL-6, which in turn promotes the growth of cancer cells at the metastatic sites (Groblewska et al., 2008). The other theory is that interleukin-6 is secreted in cancerous tumors from the blind cells that are activated (Chung & Chang, 2003). A research has shown that serum containing interleukin 6 was substantially higher in patients with colorectal cancer compared with patients with adenoma and healthy subjects (Nikiteas et al., 2005). This is consistent with our report, which gave very similar percentages of the above mentioned study,

which takes attention to the fact that our conclusions are backed by it.

Other studies have shown that the role of interleukin-6 in colon and rectal cancer has been strong, where levels have been 2-10 times higher, and this increase is greater in cases of metastasis (oşkun, 2018 ; Il'yasova et al., 2005). By binding this secreted cytokine to the membrane receptor (IL-6R) consisting of a ligand binding (GP 80) and signal transduction subunits (GP 130), this increase in interleukin-6 levels in CRC infected patients results in activation. Some cancer functions have been included (McMillan et al. ,2003).

CRP is one of the proteins in the acute phase that is secreted during infections, especially colitis and rectal infections. The role of this protein in the incidence of cancer and rectal cancer has been documented in several studies (Canna et al., 2004) have reported the relationship between CRP and its role in cancer occurrence, especially at ages over 70 years. These studies offered a clear indicator of our research, which showed results showing a rise in CRP in CRC cancer patients.

In evaluating the alternative risk relationships for the development of CRC cancer, it is clear that there are unique characteristics where the hypothesis states that inflammation plays a major role in colon carcinogenesis. The hypothesis that the risk of developing CRC can be predicted by CRP protein, but there are no specific mechanisms that explain this.

According to McMillan et al. (2003; Canna et al. (2004) studies on the role of CRP in raising the risk of colon and rectal cancer, prognosis, tumor recurrence and survival have shown that high blood CRP levels give an indication before and after the resection process, which is consistent with the results obtained by the measurement. Our analysis yields a prediction of CRC survival from this.

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