

IL-10 serum level estimation in Iraqi colorectal and gastric cancer patients

Ahmed R. Abdulla*

Ali H. Mohammed Ali*

Khudair J. Al-Rawaq**

Anas N. Ibraheem*

BVM, MSc (path), PhD Tumor Immunology

BVM, MSc (Biotechnology)

MBChB, DMRT

BSc (Microbiology)

Summary:

Background: Gastrointestinal cancers (GITc) is a worldwide problem. In Iraq, Gastric cancer is the 9th commonest of the top cancers while colorectal cancers it is considered as the 7th commonest ten cancers. IL-10 appears to be more of a pro-tumor than anti-tumor properties in both colorectal and gastric cancers.

Objective: is to estimate the serum level of IL-10 in the Iraqi colorectal and gastric cancer Patients and its relation to the progress of disease.

Patients and Methods: In our study, 54 serum samples were collected starting from the 1st of January to mid of March 2011, to investigate the IL-10 serum level by using ELISA kit. 38 colorectal and gastric cancer patients (H. Pylori +ve) and 16 of healthy control group.

Results: The results showed that IL-10 serum levels of both GIT tumors were increased significantly ($p < 0.05$) comparing with the healthy control group.

Conclusion: In conclusion, the high serum level of IL-10 in colorectal cancers is due to the pro-tumor properties of IL-10 while in gastric cancer while the association of IL-10 genotypes specifically the single nucleotide polymorphism of the IL-10 promoter region may be the source of its serum increasing level.

Keywords: IL-10, Colorectal Cancer, Gastric Cancer.

J Fac Med Baghdad
2012; Vol.54, No.2
Received Feb.2012
Accepted Apr. 2012

Introduction:

Gastrointestinal cancers (GITc) is a worldwide problem, which have ranged from about 4,500 to about 6,000 new cases in the United States each year. Gastric cancer is the 2nd most common cancer in the world, referred to as stomach cancer which causes about 800,000 deaths worldwide per year [1]. In Iraq the Gastric cancer is the 9th commonest cancer [2]. On the other hand Colorectal cancers (CRC) is 1st of the most common and aggressive types of cancer worldwide which is obviously seen in 2006 there were about 412,900 new cases of CRC in Europe and 142,672 in the United States [3] while in Iraq it is considered as the 7th commonest cancer [2]. The systemic and local cytokine environment may modulate the immunogenicity and affect anti-tumor immune function of tumor-infiltrating lymphocytes. Focusing on individual cytokines has generated evidence that pro-inflammatory cytokine and anti-inflammatory cytokines may have a complex role in gastrointestinal carcinogenesis [4]. IL-10 is an immunoregulatory cytokine and its main biological function is limitation and termination of inflammatory responses. IL-10 also regulates differentiation and proliferation of several immune cells [5]. Antiangiogenic properties of IL-10 have also been described. Thus, its dual

role as immunosuppressive and Antiangiogenic cytokine may have both promoting and inhibiting effect on tumor development and progression [6]. The role of IL-10 in CRC is inhibiting the expression of p40, and both p35 and p19 subunits in macrophages and dendrite cells [7] while in GC, IL-10 mRNA high expression and elevated serum level are correlated with advanced stage and progress of disease [8]. The aim of our study is to estimate the serum level of IL-10 in gastric and colorectal cancer patients.

Patients and methods:

Sample collection: Samples of blood from 38 colorectal and gastric cancer patient (H. Pylori +ve) and 16 healthy individuals were collected [after the definite diagnosis and before taking the chemotherapy] from the Oncology clinic / Baghdad Teaching Hospital and the teaching hospital for the GIT and liver diseases / medical city starting from the 1st of January to the mid of March 2011 and sent for (IL-10) estimation. Histopathological reports were obtained from the patients for definite diagnosis. 5-10 ml of venous blood was drawn from the patients to gain serum for the detection of Human Interleukin 10 (IL-10) by using ELISA technique. **IL-10 ELISA Kit:** The microtiter plate has been pre-coated with an antibody specific to IL-10 then standards and samples were added to the appropriate microtiter plate

*Biotechnology Research Center / Al-Nahrain University.

**Dept. of Surgery, Medical College / University of Baghdad.

wells .a biotin conjugated antibody preparation specific for IL-10 and avidin conjugated to Horseradish peroxidase (HRP) is added to each microtiter plate wells .Incubation. Occurred then TMB (3,3',5,5'-tetramethyl-benzidine) substrate solution was added to each well. only those wells that contain IL-10 biotin-conjugated antibody avidin will exhibit a change in color. The enzyme substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of $450 \text{ nm} \pm 2 \text{ nm}$. The concentration of IL-10

in the samples is then determined by comparing the O.D. of the samples to the standard curve. Statistical analysis was performed using T-test with significant difference ($P < 0.05$).

Results:

The demographic data showed that 90% of the colorectal and gastric cancer type was adenocarcinoma (Figure 3) and other data can be seen in figures 1 and 2.

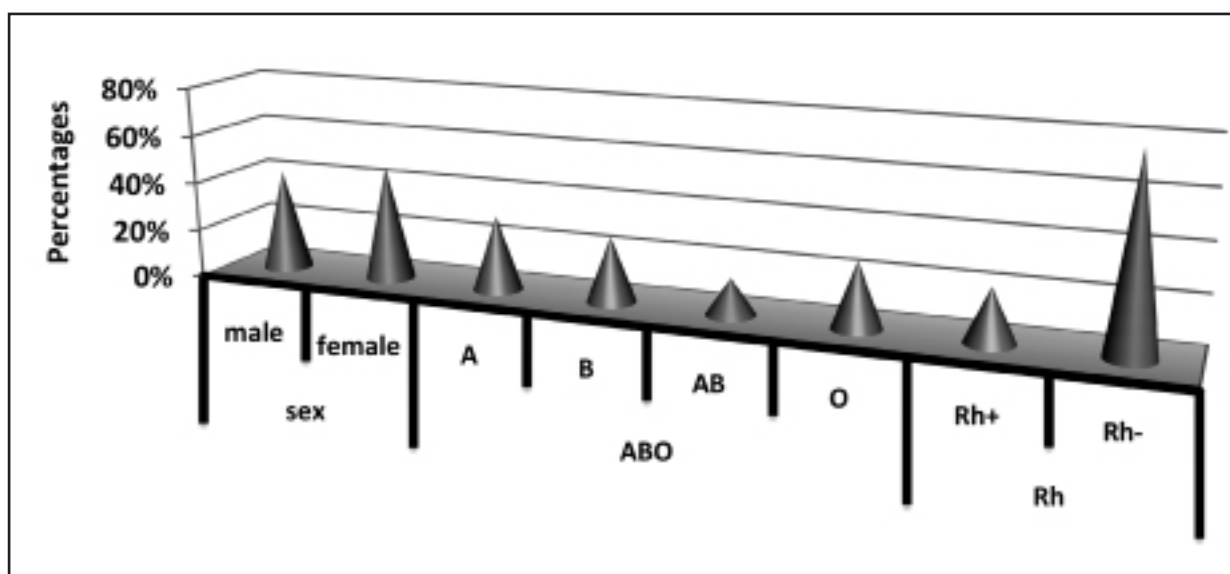


Figure (1): data showed sex, ABO system and Rh.

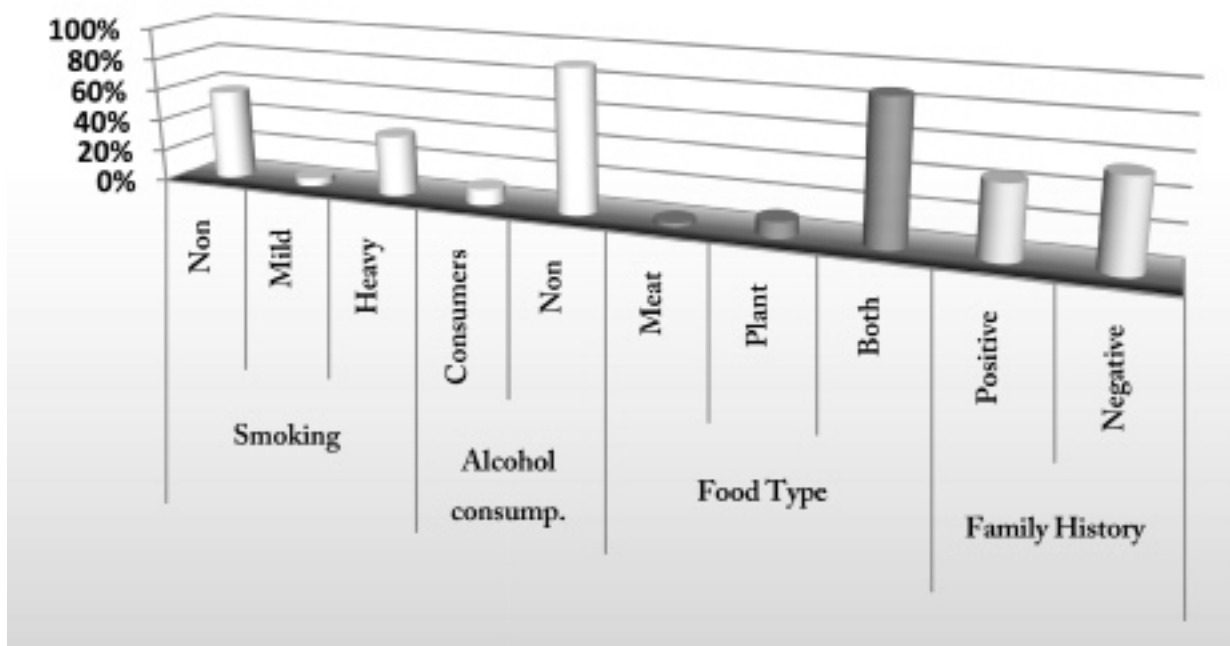


Figure (2): it shows smoking ,alcohol conception , food intake and Family History

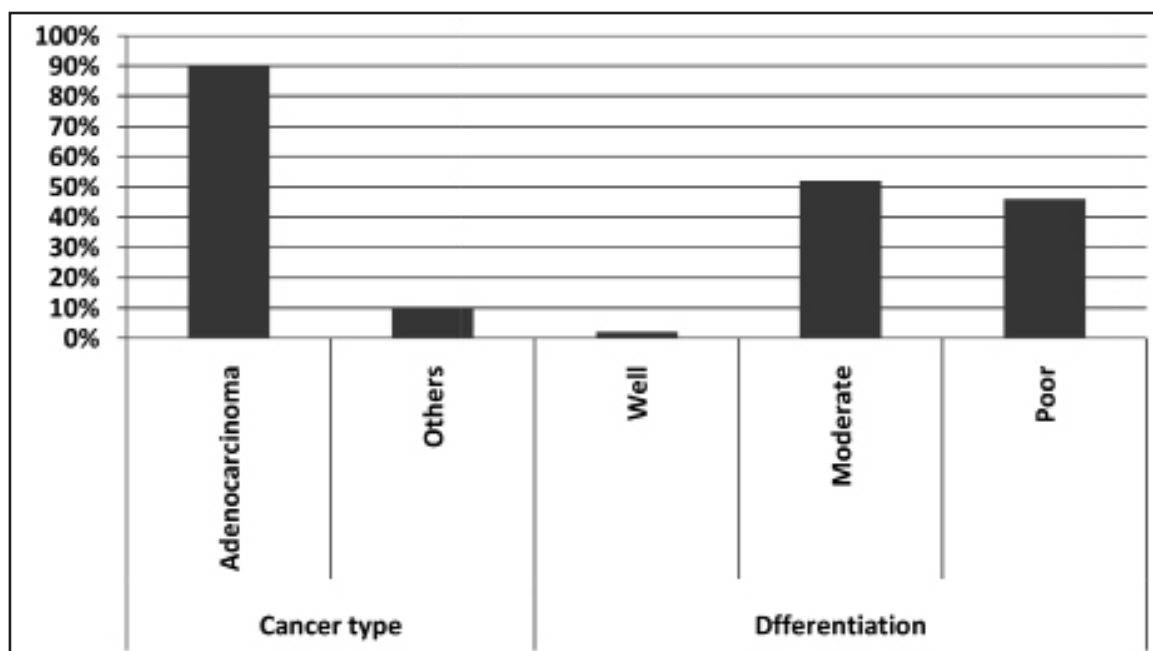


Figure (3): it shows the cancer type&Differentiation.

Serum levels of IL-10 of GIT tumors (colorectal & gastric) and healthy donor's were analyzed, the results showed that IL-10 serum levels of both GIT tumors were increased significantly ($p < 0.05$) comparing with the healthy control group. (Table1& Figure6).

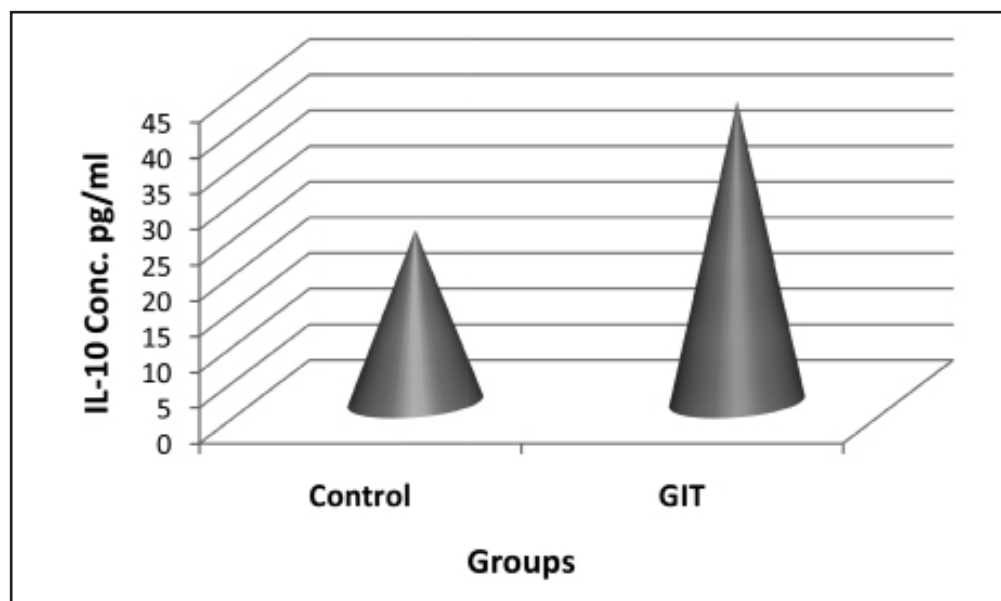


Figure (4): IL-10 serum levels of both GIT tumors and control group.

Table (1): The significant increase of IL-10 serum levels for both GIT tumors (colorectal and gastric) compared to the healthy control group.

GIT tumors(mean ±SE)	Healthy subjects (mean ±SE)
A*	B
42.188±7.6	24.093±3.09

*Differences between A& B are significant ($P < 0.05$) to compare columns

Discussion:

As it is known that IL-10 is Th2- cytokine, which increases antibody synthesis, promotes the humoral immune response and suppresses the antitumor immunity, demonstrated by Stanilovet al.2010 [9] who showed that Functional antagonist of IL-12p70 is the IL-10 which was of high level in the serum of a 48 colorectal cancer patients.IL-10 appears to be more of a pro-tumor than anti-tumor properties. The pro-tumor properties of IL-10 can be explained by the inhibitory effect

on the Th1-cytokine production, in particular IL-12p70 [10], its inhibitory effect by engaging apoptosis and stimulation of cell proliferation [11]. Also, there is evidence that the tumor infiltrated lymphocytes inside the tumor mass are not effective because some tumor cells secrete IL-10. IL-10 secretion is one of the mechanisms with which the tumor cells "avoid" the immunological surveillance which at the end will also associate to raise the IL-10 serum level [11, 12] which can explain the significant increased level of serum IL-10 ($p < 0.05$) in our study. The pivotal roles of different cytokines in regulating antimicrobial immunity and inflammation make them attractive candidates for being genetic host markers in evaluating individual susceptibility to Gastric Cancer development [13]. They may influence the risk of developing Gastric Cancer by altering the quality and vigor of inflammatory responses produced by the host after exposure to various environmental or infectious triggers [14] that is seen in our study in all the cases of Gastric tumors were H. Pylori +ve. The significant increase ($P < 0.05$) of the IL10 serum level may be because the association of IL-10 genotypes (single nucleotide polymorphism) with Gastric Cancers appears to be biologically and clinically important. IL-10 is a key immunosuppressive cytokine that gears the immune response towards a Th2 cell response [15]. The haplotype alleles formed in the promoter region of the IL-10 gene at positions -1082, -819 and -592 are related to high IL-10 producing capability. Compared with the ATA haplotype, the GCC haplotype is associated with a higher production of IL-10 in culture of stimulated peripheral blood mononuclear cells [16, 17]. Such IL-10 haplotypes are linked to susceptibility and severity of Gastric Cancers. The finding that there was an increased risk of Gastric Cancers in high IL-10 producer haplotype was in agreement with the concept that Th2 cytokines including IL-10 are highly expressed in patients with Gastric Cancers as was shown in our study results [18, 19, 20] and This notion could partly be explained by reported findings that increased expression of mRNA and elevated serum levels of IL-10 are correlated with the progression of Gastric Cancers [21, 22]. In conclusion, the increased IL10 serum level in colorectal cancers may be due to the Functional antagonism of IL-10 toward IL-12p70 which will cause more IL10 secretion and may be the secretion of this cytokine by the tumor cell itself to modulate the Immune system towards Th2 rather than Th1. While in gastric cancers the association of IL-10 genotypes with Gastric Cancers specifically the single nucleotide polymorphism of the IL10 promoter region may be the cause of such serum elevation.

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