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IL-17 (gene expression) as a new biological marker for diagnosing the gastric and colorectal cancer

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***Abstract*---**Background: Malignant gastrointestinal tumors such as esophageal, gastric, liver, colorectal, and pancreatic carcinomas are a major cause of cancer-related deaths worldwide proinflammatory cytokine interleukin-17 (IL-17) produced by Th17 cells, a T helper cell subset developed from an activated CD4+ T cell. IL-17 plays a potent role in T-cell mediated angiogenesis, promotes carcinogenesis and it has effects on immune resistance, proliferation and metastasis. This study was aimed to investicate the Serum IL-17 levels in Iraqi patients with GC and CRC. Materials and methods: peripheral Blood samples were collected from 30 Iraqi patient divided in to two groups 14women and 16 men with age rang 37-57 years, thay attended Gastroenterology and liver teaching hospital in Baghdad city and diagnosed with early detected (early onest) GC and CRC, and 30 healthy women and men were matched with patients as a control. IL-17 serum levels were measured using the highly sensitive Elisa kits (Human IL-17A Mini ABTS ELISA Development Kit , USA) Results:. Elisa assay demonstrated that IL-17 levels were highly expressed in GC and CRC patients, more especially in patients with early detected (early onset) disease. the serum level of IL-17 for the understudying groups, were for the patients groups (2.194 ±0.03 pg/ml) as compared to control (1.462 ± 0.04 pg/ml) there was a significant difference under ($p<0.05$). Conclusion: : Since serum IL-17 levels were significantly higher in patients with GC and CRC compared to health subjects. Serum IL-17 levels may be a new candidate marker in the diagnosis GC and CRC at early detected (early onest).

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***Keywords*---**serum, interleukin-17, gastric, colorectal cancer, diagnosis, marker.

Introduction

Malignant gastrointestinal tumors such as esophageal, gastric, liver, colorectal, and pancreatic carcinomas are a major cause of cancer-related deaths worldwide [1]. GCis the fourth most common cancer in the world and occupies the second position regarding cancer-related deaths [2,3].The World Health Organization estimates that in 2018, GC accounted for 783,000 deaths worldwide[4]Over the past decade a slight decline has been observed in CRC cancer (CRC), however; it still remains the third most common cancer among cancer patients worldwide[5] In 2018,nearly 2.0 millionnewly diagnosed CRC cases and more than 0.8 million related deaths are expected to occur worldwide [4].According to the latest WHO data published in 2018 Stomach Cancer Deaths in Iraq reached 966 or 0.56% of total deaths and death rate of CRC cancer 6.30%[4]

Several Iraqi studies were highlight on genetic effect in inflammatory bowel disease and colorectal cancer , Fadhe and Mahood, were reported to important role of *TNF*-α (-1031) gene polymorphisms in promoter region in the etiology e of inflammatory bowel disease of Iraqi population especially in Crohn disease patients [6] , PTEN gene mutations may cause the etiology of CRC as there were genetic alterations in bowel inflammation and CRC patients [7]. Other Iraqi study results revealed no mutation in position 1799 for exon fifteen in both samples of bowel inflammation and CRC cancer [8]. Recently, it has been demonstrated that, among a group of “inflammation-related” neoplasms, including GI malignancies, the action of proinflammatory cytokine interleukin-17 (IL-17), may be of particular significance [9-11].

IL-17 plays a potent role in T-cell mediated angiogenesis, promotes carcinogenesis and it has effects on immune resistance, proliferation and metastasis. IL-17 is predominantly produced and secreted by activated CD4 T-cells [12] It should be noted that IL-17 is also produced by other sources such as natural killer cells (NK), CD8+ T cells, neutrophils, eosinophils, macrophages, dendritic cells and gamma delta T cells (γδT) [13,14]. Regulatory T (Treg) cells, generally known as immune suppressors, also produce IL-17[15]. Moreover, recently, IL-17 emerged to have an outstanding performance against cancer and the role of Th17 cells in malignancy is still under discussion [16]. IL-17 expression is elevated in different human tumors, such as cervical cancer, hepatocellular carcinoma, ovarian cancer, esophageal cancer, breast cancer, GC cancer and CRC [16,17]. It was shown that IL-17 can trigger some cancer pathways such as Src/PI3K/Akt/nuclear factor-κB (NFκB), MAPK, Stat3 and COX-2. These pathways have roles in tumorigenesis, angiogenesis and metastasis [17]. Increased understanding of the biology of IL-17 has revealed that this cytokine is a central player in immunity at the sites most exposed to microorganisms. Although it has been strongly associated with immunopathology, IL-17 also has an important role in host defense , so this makes it more important in GC and CRC[18]

Materials and Methods

Subjects

Tow sets of blood samples have been collected, the first set consisted of 30 subjects of patients with cancer in some parts of gastroitestinal particularly GC and CRC , they attended Gastroenterology and liver teaching hospital in Baghdad city, for follow up. the second set a control group consisted of 30 subjects apparently healthy, and their age was matching to patients group. All examined subjects were residence in Baghdad city. The volume of 2.800 ml of peripheral blood samples was collected by disposable syringe and transferred into gel tube left for half an hour, then centrifuged for 15 minutes at 3000 RPM, serum was transferred into 1.5ml Eppendorf tubes and stored at -20ºC for serological study. The serological detection was carried out by using ELISA technique.

Measurement of serum IL-17 levels

Measurement of serum IL-17 levels serum samples were obtained on first admission before any treatment was given to patients and the serum specimens were collected following centrifugation (10 min at 4000 rpm) at room temperature and frozen immediately at -80°C until analysis. Enzyme-linked immunosorbent assay (ELISA) (Human IL-17A Mini ABTS ELISA Development Kit , USA) was used (a double-antibody sandwich ELISA) to determine the level of human IL17 in samples. Serum samples were placed to the wells which were precoated with human IL-17 monoclonal antibody. Following incubation, IL-17 antibodies labeled with biotin and combined with streptavidin-HRP were added to form immune complex and allowed to incubate for 2 h. Unbound material was washed away, and then, chromogen solution was added for the conversion of the colorless solution to a blue solution (5-15 min), the intensity of which was proportional to the amount of IL-17 in the sample. Under the effect of the acidic stop IL-17 in the diagnosis of GC 1603 JBUON 2019; 24(4): 1603 solution, the color became yellow. The colored reaction product was measured using an automated ELISA reader (ChroMate® 4300 Microplate Awareness Technology). The results were expressed as pg/mL.

Statistical analyses

The Statistical Analysis System- SAS (2012) program was used for outcome of different factors in study parameters. Least significant difference –LSD test (ANOVA) was used to significant compare between means of parameters in this study (19). WINPEPI computer programs (version 11.63) was used to calculate the statistical significance of P-value

Results

Interleukin-17 (IL-17) serum level

As shown in (Table 1) which illustrated the serum level of IL-17 in 30 patients and 30 controls. There was a high significant elevation in IL-17 patients serum level. The result was presented on (means ± SE) pg/ml and it was (2.194 ± 0.03) pg/ml

in patient groups as compared with control groups which recorded (1.462 ± 0.04) pg/ml under (P<0.01).

Table 1: Comparison between patients and control in IL-17 serum level

Group	No	Mean ± SE of IL-17
Patients	30	2.194 ± 0.03
Control	30	1.462 ± 0.04
T-test	---	0.108 **
P-value	---	0.0001
** (P<0.01).		

All serum samples were collected from early dignosed patient with GI cancer which included GC and CRC . IL-17 serum level showed an elevation level in patients as compared to healthy controls. As shown in (Figure 1) which illustrated the serum level of IL-17 in patients and controls The result was presented on (means ± SE) pg/ml and it was (2.194 ± 0.03) pg/ml in patient groups as compared with control groups which recorded (1.462 ± 0.04) pg/ml. There was a high significant elevation in IL-17 patients serum level under (P<0.01).

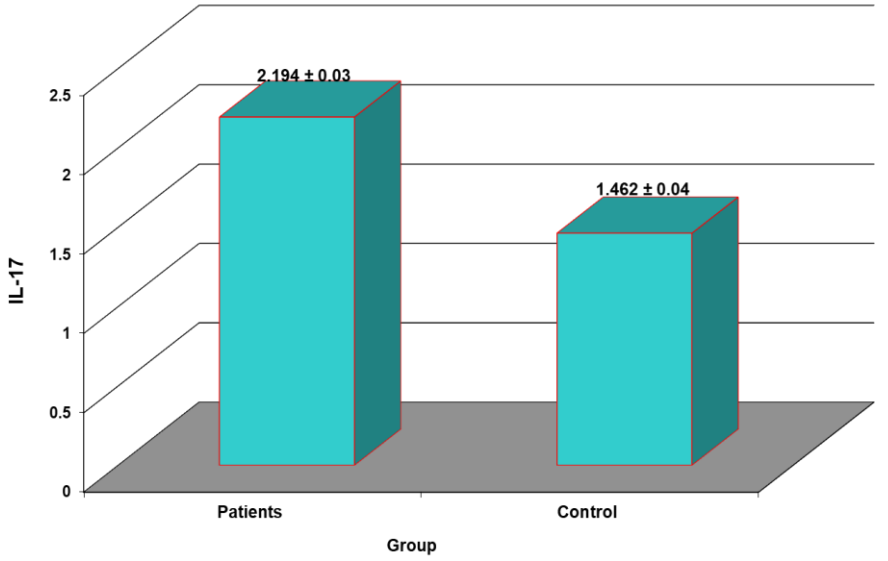

Figure 1. Comparison between patients and control in IL-17

Relationship between age and gender with IL-17 of patients

The current study results as shown in (Table 2) showed significant differences in mean ± SE of IL-17 for all studied age grupes of patient, with an age range 37-57 years, the means of age groups (<40, 40-50, >50) were (2.17 ± 0.05, 2.32 ± 0.06, 2.07 ± 0.04), respectively,with LSD value 0.181 and p-value 0.0451, while there was non-significant differences in gender, the means were (2.22 ± 0.04, 2.16 ± 0.06) in male and female respectively with LSD value 0.142 and p-value 0.459.

Table 2: Relationship between age and gender with IL-17 of patients

Characters	Level	Mean ± SE of IL-17	LSD value	P-value
Age (year)	< 40	2.17 ± 0.05	0.181 *	0.0451
	-40-50-	2.32 ± 0.06		
	> 50	2.07 ± 0.04		
Gender	Male	2.22 ± 0.04	0.142 NS	0.459
	Female	2.16 ± 0.06		
* (P<0.05), NS: Non-Significant.				

Discussion

Among numerous cytokines one of the most investigated that has role in both hallmarks of cancer is the recently described IL-17 [20]. In a new study, IL-17 was found to be positively correlated with the transformation of quiescent GC stem cells into invasive GC stem cells and the investigators concluded that targeting IL-17 may emerge as a possible novel therapeutic strategy for GC [21]. Various genetic variants of IL-17 are likely to be associated with risk of GC in Chinese population [22]. Signaling by IL-17A activates the NFκB, AP1, and C/EBP pathways leading to cancer progression [23]. Analyses of IL-17 expression have shown that patients with diffuse GC had significantly lower expression of IL-17 in comparison to patients with intestinal type [23]. In all these published studies, it is seen that IL-17 has an emerging role in GC. In our study, we found that patients with early detected (early onest) GC and CRC had significantly higher levels of IL-17 than the control group. There was a similar findings with us which carried out by [24] which showed that patients with GC had higher levels of IL-17 in serum, this result agreed with [25]who analyses revealed that, in comparison to healthy individuals, patients with early gastric carcinoma had significantly higher mean values of IL-17 and agree with the study of us.

Higher expression of IL-17 in tissue and serum of patients with CRC suggests that this cytokine could be used as a prognostic marker in CRC [26]. IL-17 promote angiogenesis [27] and Prostaglandin E2 (PGE2) production, which both lead to the better survival of CRC cells [28]. [29] have shown that in tumor bearing mice, PGE2 suppresses the cytotoxicity and cytokine production of natural killer (NK) cells via EP4 signaling. Furthermore, the polarization of tumor-associated macrophages (TAMs) towards tumor-promoting M2 macrophages is also influenced by PGE2 in lung carcinoma cells [30]. Interestingly, Liu et al. [30] also reported that IL-17 is important in recruiting macrophages to the tumor microenvironment prior to their polarization, demonstrating a cooperative effect between IL-17 and PGE2. This conclusion is supported by an abundant literature showing colon cancer protection by nonsteroidal anti-inflammatory drugs (NSAIDs), the overwhelmingly positive results associated with cyclooxygenase-2 (COX-2) enzymes.or microsomal PGE synthase-1 (mPGES-1) deletion in pre-clinical mouse tumor models, and the efficacy of targeting EP [31]. A study by [32] reported increased level of IL-17 is associated with poor prognosis of CRC. Additionally, Liu et al. showed that 5 year survival of 26 patients with lower IL-17 level was 72.41% and it was 38.08% in 26 patients with higher IL-17 level [27]. Hyun et al. demonstrated that the incidence of colitis associated colon cancer is

significantly decreased in IL-17 deficient mice [33]. However, a study by [34] showed that IL17 expression is associated with good histological differentiation and early Duke's stage. Also, Kaplan-Meier analysis represented that survival rates were longer in patients with high zer level of IL-17 and therefore, they suggested that elevated level of IL-17 contributes to good prognosis in patients with CRC and it might be a protective agent in CRC progression. These results are different from the results of Liu's study. This is perhaps due to the fact that Lin et al. included patients with different stages of the CRC but Liu et al. only included patients with stage III CRC [34]. Published data demonstrated that the level of IL-17 was significantly higher in CRC tissues [34–36]. Cui et al. have shown that the up-regulation of IL-17 begins from adenoma stage and its level is higher at the cancer stage[37].

Recently,the study of [38]showed serum IL-17A levels in early stage CRC patients were signifcantly higher than those in the controls whil the study of [39] showed serum IL-17A levels in advanced stage GC patients were signifcantly higher than those in the controls, these studies were agreement with our study in elevated serum level of IL-17 but we resulted that in early detected (early onest) disease of GC and CRC.

Conclusions

In conclusion, although we were not able to demonstrate the effect of IL-17 on survival and clinical course, the significant elevation of IL-17 in GC and CRC compared to healthy individuals suggests that IL-17 may be a candidate as diagnostic marker for GC and CRC. Future studies will provide a better understanding of this molecule.

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