

## Increasing serum level of transforming growth factor beta 2 (TGF- $\beta$ ) in patients with colorectal and gastric cancer in Iraq

إرتفاع مستوى Transforming growth factor beta2 (TGF- $\beta$ ) في مصل مرضى سرطانات القولون والمستقيم والمعدة في العراق

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### Abstract

Gastrointestinal cancers (GITs) are worldwide problem particularly in highly developed countries. In Iraq, gastric cancer (GC) is the 9th most common cancer while colorectal cancers (CRC) is considered as the 7th most common cancer among all cancer patients in both males and females. The Objective of this study was to estimate the serum level of transforming growth factor beta 2 (TGF- $\beta$ ) in Iraqi patients who are complying from gastric and colorectal cancers. Fifty four serum samples were collected starting from the 1st of March till the mid of May of 2011 to investigate the TGF- $\beta$  serum level by using ELISA technique. Thirty eight samples were gastric (*H.pylori* +ve) and colorectal cancer patients (GC=17, CRC=21) while the other 16 samples considered as a healthy control group. The results showed that TGF- $\beta$  serum levels of both GIT tumors were increased significantly ( $p<0.05$ ) compared to the healthy control group. In conclusion, the presented study showed elevated serum level of TGF- $\beta$  in Gastric cancer patients which could point out to use this elevation as a biomarker for tumor prognosis; while in colorectal cancer it may evade the immune system cancer killing mechanisms. We recommend further studies concerning the correlation between serum level of TGF- $\beta$  in Gastric cancer patients with staging and grading, and more immunological techniques can be implied to know the exact immunological evading mechanism of colorectal cancer cells.

### المستخلص

يعتبر سرطان الجهاز الهضمي من أكثر المشاكل الصحية في العالم خاصة في البلدان المتقدمة أو ما يسمى ببلدان العالم الأول. أما في العراق وحسب احصائيات مجلس السرطان التابع لوزارة الصحة فيحتل سرطان المعدة الترتيب التاسع وسرطان القولون والمستقيم الترتيب السابع ضمن قائمة العشر اورام الأكثر انتشارا بين الرجال والنساء المصابين بمرض السرطان. كان الهدف من هذه الدراسة هو قياس مستوى TGF- $\beta$  في مصل المرضى العراقيين المصابين بسرطان المعدة والقولون والمستقيم، وقد جمعت 54 عينة مصل ابتداء من الأول من آذار وانتهاء بمنتصف مايس من سنة 2011 لقياس مستوى TGF- $\beta$  باستخدام تقنية الاليزا ELISA، 38 منها كانت لمرضى سرطان المعدة (*H.pylori* +ve) وسرطان القولون والمستقيم (سرطان المعدة: 17 حالة، سرطان القولون والمستقيم: 21 حالة) و الباقى (16 عينة) تم جمعها من أفراد أصحاء

Key words :Transforming growth factor beta (TGF- $\beta$ ), Gastric cancer (GC), Colorectal cancer (CRC)

رجال ونساء والتي اعتبرت مجموعة سيطرة . أظهرت النتائج ارتفاعا في مستوى TGF- $\beta$  في مصل المرضى المصابين بكل نوعي السرطان وبشكل معنوي ( $p < 0.05$ ) مقارنة بمجموعة السيطرة . من خلال هذه الدراسة استنتجنا ان ارتفاع مستوى TGF- $\beta$  في مصل مرضى سرطان المعدة قد يعكس مآل سيء او خطير لحالة المريض بالمقارنة مع الدراسات الدولية المشابهة ، بينما في سرطان القولون والمستقيم قد تكون هي احدى آليات هروب الخلايا السرطانية من الفعاليات القاتلة لجهاز المناعي .

## Introduction

Gastrointestinal cancers (GITs) are a worldwide problem; about 4,500 to 6,000 new cases are registered in the United States each year [1]. Gastric cancer (GC) is the 4th most common cancer in the world and it's more common in men causes about 800,000 deaths worldwide every year [1]. In Iraq the GC is the 9th most common cancer among all cancer patients [2].

On the other hand, colorectal cancers (CRC) are considered as the 1st most common and aggressive type of cancer worldwide. About 4500-6000 new cases are registered in USA/year while in 2006 there were about 412900 new CRC in Europe [3]. In Iraq CRC is considered as the 7th most common cancer type in both males and females[1].

The systemic and local cytokines environment may modulate the immunogenicity and affect anti-tumor immune function of tumor-infiltrating lymphocytes. Focusing on individual cytokine has generated evidences that pro-inflammatory and anti-inflammatory cytokines may have a complex role in gastrointestinal carcinogenesis[4]. TGF- $\beta$  is a member of the large family of disulfide-bonded cytokines and it is a potent growth inhibitor of most types of cells [5]. It is a well-known cytokine that plays an important role in tumor progression, invasion and metastasis as well as actin cytoskeleton reorganization when it's exploited by the tumor cells in the tumor microenvironment [6].

In Gastric Cancers, TGF- $\beta$  pathway plays a key role in the suppression of gastric carcinoma in the early stages; biological signals for TGF- $\beta$  are transferred through trans-membrane serine/threonine kinase receptors, which in turn give signal to Smad proteins. Inactivation of the TGF- $\beta$  pathway often occurs in malignancies of the gastrointestinal system, including gastric cancer [7], while in many colorectal cancers (CRC); cancer cells escape the tumor-suppressor effects of TGF- $\beta$  as well as TGF- $\beta$ -induced growth inhibition. However, during the late stages of colorectal carcinogenesis, TGF- $\beta$  acts as a tumor promoter and are usually highly expressed [8].

The aim of the present study was to estimate the serum level of TGF- $\beta$ 2 in GC and CRC patients and discussing the data with the international findings to be applied for Iraqi patients.

## Materials and methods

### Sample collection

Blood Samples (5-10)ml from veins at cubital fossa of thirty eight patients with GC (*H.pylori* +ve as in histopathological reports) and CRC patients (GC=17, CRC=21) and 16 healthy individuals were collected (after definitive diagnosis and before taking the chemotherapy) at the Oncology clinic/ Baghdad Teaching Hospital and at the Teaching Hospital for the GIT and liver diseases / Medical city Starting from the 1<sup>st</sup> of January till the mid of March 2011. A questionnaire was made to obtain the demographic data (name, address, gender, ABO, Rh, tobacco smoking, Alcohol

consumption, food type and family history), while the histopathological data (cancer type, staging and grading) were taken from the patient's files. Blood samples were centrifuged at 2000 g for (10-15) minutes to obtain serum used to detect TGF- $\beta$ 2 levels by ELISA technique.

#### **Detection of TGF- $\beta$ 2 by ELISA:**

ELISA kit (Cell Singling Technology, Denver, MA, USA), was used according to the manufacturer's instructions. Briefly, the microtiter plate was pre-coated with an antibody specific to TGF- $\beta$ 2 then standards and samples were added to the appropriate microtiter plate wells. A biotin conjugated antibody preparation specific for TGF- $\beta$ 2 and avidin conjugated to Horseradish peroxidase (HRP) were added to each well. After incubation, 3, 3', 5, 5'-tetramethyl-benzidine (TMB) substrate solution was added to all wells. Only those wells that contain TGF- $\beta$ 2 biotin-conjugated antibody avidin will exhibit a change in color. The enzyme substrate reaction was terminated by adding of 3 M sulphuric acid solution then the color change was measured spectrophotometrically (ASYS, Australia) at a wavelength of 450 nm  $\pm$  2 nm. Finally, TGF- $\beta$ 2 concentration was determined by comparing the optical density (O.D.) of each sample to the standard curve.

#### **Statistical Analysis**

Statistical analysis was performed using student's T-test (Microsoft office Excel worksheet, Microsoft Company, USA). Data were considered significant at  $P < 0.05$ .

#### **Results**

##### **Demographic and Histopathological Data**

The demographic data showed that the mean age of the patients was 55.6 year, their gender were 16 males 42% and 22 females 58%, their ABO system was categorized as the following: A 12 (31%), B 10(27%), AB 6 (15%) and O 10 (27%), while the Rh factor was positive in 8 (22%) and negative in 30 (78%) Figure (1).

Patients who were non tobacco smokers were 22(57%), mild tobacco smoker (less than 10cigareet/day) were 2(5%) and heavily tobacco smokers (more than 10 cigarette/day) were 14 (38%), alcohol consumers were 4 (10%) and non-alcohol consumers were 34(90%), patients with food mainly vegetarian were 4 (10%), meat 1 (3%) and mixed diet were 33 (87%). Family history (1<sup>st</sup>, 2<sup>nd</sup> & 3<sup>rd</sup> degree) was positive in 17(45%), patients who 7(41%) have relatives suffer from GIT cancers while the rest 10(59%) have relatives that suffer from other organs cancers Figure (2). The histopathological data showed that the cancer types were adenocarcinomas 36(90%) and others 2(10%) were diagnosed as signet ring and mucinous carcinomas Figure (3). Tumor differentiation was classified as: well differentiated 1(2%), moderately differentiated 20 (52%) and poorly differentiated 17 (46%).

##### **Serum levels of TGF- $\beta$ 2**

The results showed that TGF- $\beta$ 2 serum levels of both GIT tumors were increased significantly ( $p < 0.05$ ) comparing with the healthy control group. Corresponding figures were  $41.928 \pm 9.239$  SE in GIT cancers patients and  $18.5 \pm 2.589$  SE in healthy controls Figure (4).

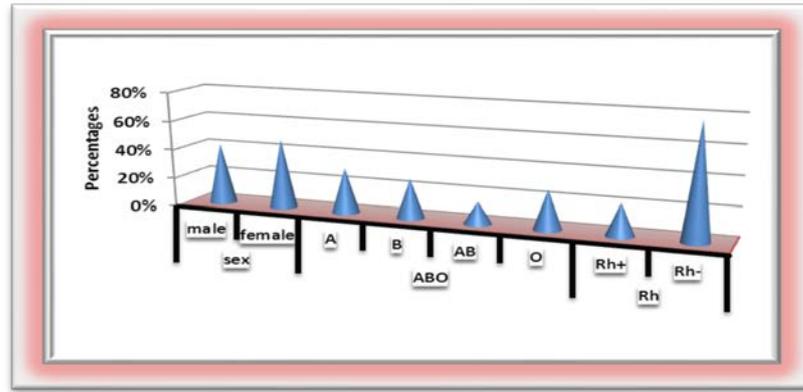


Fig (1): gender, ABO system and Rh data of the 38 patients with GIT cancers

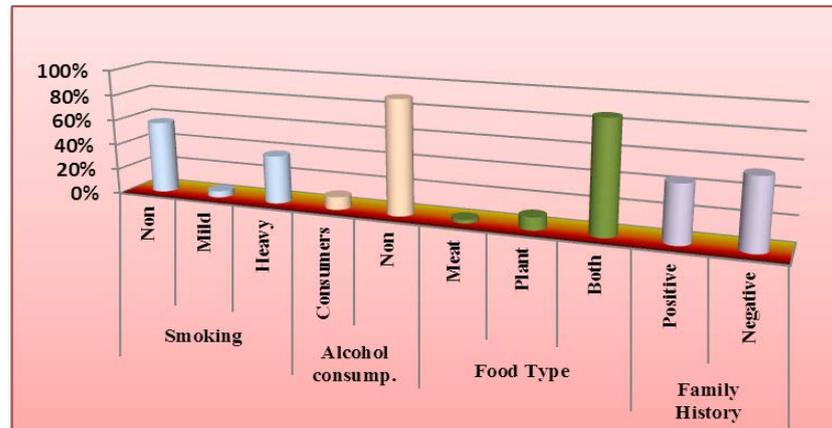


Fig (2): Data of tobacco smoking, alcohol consumption, food intake and family history of 38 GIT cancers

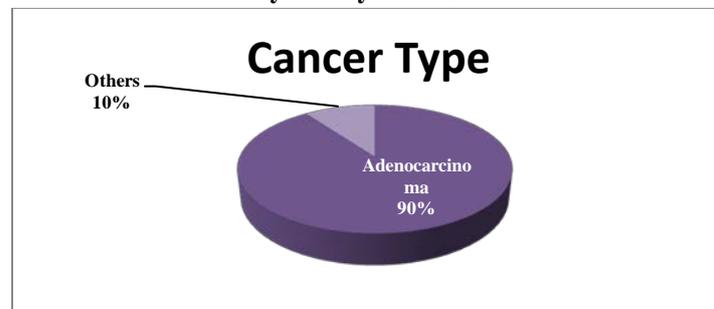


Fig (3): Histological types of GIT cancers patients in 38 patients

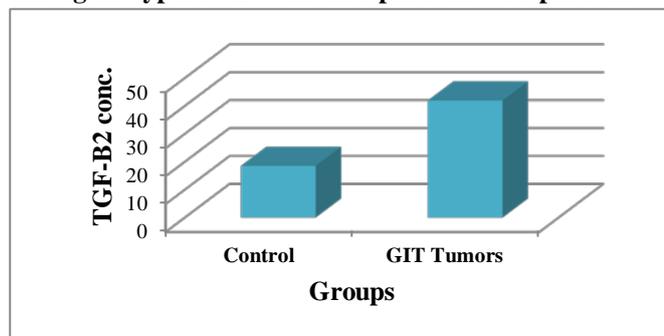


Fig (4): TGF-β2 serum levels of both GIT tumors and control group blood samples

## Discussion

All transforming growth factor beta (TGF- $\beta$ ) isoforms which (1, 2&3) share sequence homology and similar functions [9, 10]. TGF- $\beta$  signals have an important role in the metastatic spread of cancer cells [9, 10, 11], such as migration, invasion, and epithelial-to-mesenchymal transition (EMT) [10, 12]. TGF- $\beta$ 2 has been found to be overexpressed locally in many deep invasive primary tumors which correlated with lymph node metastasis and poor prognosis of gastric and colorectal cancers [13, 14, 15]. Updated data suggest that the main source for TGF- $\beta$ 2 could be from cancer stem cells [16].

Elevated levels of TGF- $\beta$ 1 and TGF- $\beta$ 2 in GC patients may reflect or, because of the potent immunosuppressive properties of TGF- $\beta$ , and may be even causatively involved in the impairment of the immune system in melanoma patients with advanced tumor dissemination as a model for other tumors. A highly immunogenic tumor transfected with a murine TGF- $\beta$ 1 cDNA escaped immune surveillance in mice, and TGF- $\beta$ 2 has been identified as the T-cell suppressor factor of human glioblastoma [17, 18]. Furthermore, treatment of mice with anti-TGF- $\beta$  antibodies inhibited growth of human breast cancer cells by enhancing spleen NK-cell activity [19]. In addition, B16 melanoma growth and metastasis in vivo was inhibited by treatment of mice with anti-transforming growth factor beta antibody and interleukin 2 [20], indicating that therapeutic interventions for blocking systemic TGF- $\beta$  overexpression should be taken under further consideration.

The latent complex of TGF- $\beta$  is not biologically active and has a longer half-life and volume of distribution than the mature forms. The precursor peptide appears to have a decreased potency to inhibit cell growth compared to the mature TGF- $\beta$  [21]. As such, the precursor peptide may provide an “intermediate” level of activity between latent and mature forms. Because a wide variety of cell types are responsive to TGF- $\beta$ , this broad spectrum of forms may provide a better control over the actions of cells by TGF- $\beta$ . At the same time, the spectrum of TGF- $\beta$  forms provides more potential sites for control of TGF- $\beta$  secretion by exogenous substances such as TNF- $\alpha$  [22].

In some clinical studies, elevated serum TGF- $\beta$  levels have been observed in gastric cancer patients with poor prognoses [23], those in an advanced stage, or those with poorly differentiated or invasive type adenocarcinoma [24], and this is may be compatible to the results of this study. The source of this elevation has not been identified; therefore, TGF- $\beta$  produced by cancer cells probably functions in an autocrine and paracrine manner only in the microenvironment in the tumor tissues of gastric cancer. Although the latent form of TGF- $\beta$  is activated to active form extracellularly, active TGF- $\beta$  was unlikely to be present in plasma because this form has a short half-life in the blood circulation [25].

In colorectal cancer, serum TGF- $\beta$ 1 and 2 [26, 27] and Vascular Endothelial Growth Factor (VEGF) [28, 29] levels were increased significantly which also seen in this study for TGF- $\beta$ 2. In some studies the correlation of serum TGF- $\beta$ 1 levels with liver metastasis volume and the reported association between plasma TGF- $\beta$ 1 level and tumor mRNA expression [30] are consistent with the tumor being a source of TGF-

$\beta$ 1. In CRC patients the elevation of TGF- $\beta$ 1 in serum suppresses mature DC differentiation in murine marrow culture [31] and prevents Langerhans cell maturation [32] which may be its one of the immune evading mechanisms that is done by tumor cells. The elevated serum levels of TGF- $\beta$ 2 and over-expression of TGF- $\beta$ 2 in colorectal cancer tissue were correlated significantly with invasion and metastasis of colorectal cancer [33].

In conclusion, the presented data showed elevated serum level of TGF- $\beta$  in GC patients which could point out to use this elevation as a biomarker for tumor prognosis, while in CRC it's may be to evade the immune cancer killing mechanisms. We recommend further studies concerning the correlation between serum levels of TGF- $\beta$  in Gastric cancer patients with staging and grading as well as more immunological techniques can be implied to know the exact immunological evading mechanism of colorectal cancer cells.

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