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Teratocarcinoma-Derived Growth Factor-1 (*TDGF-1*): A Guide Molecule for Monitoring the Treatment Success of Iraqi Patients with Colorectal Carcinoma

Abdul-Rahman M. Geeran Al-Fahdawi[1], Mohammed A.Hamad[2], Eman M.Rasheed Al-Hadithy[3]

[1]Department of human Anatomy , College Of Medicine/ University of Anbar, Al-Anbar31002,Iraq,
[2] Department of Biotechnology , College of Applied science, University of Fallujah, Al-Anbar31002, Iraq,
[3]Department of Physiology, College of Medicine/ University of Fallujah, Al-Anbar31002,Iraq.

Abstract

This study was carried out in Al Ramadi Teaching hospital from the period March 2013 till August 2013 on sixty two (62) Iraqi patients with colorectal carcinoma. Their ages range from 8-47 years old complain from disturbances in GIT . All patients were visiting the department of surgery in Al-Ramadi teaching hospital. Five ml venous blood was withdrawn from the patients and placed into a plain test tube without anticoagulant to obtain sera . The blood samples were taken from the patients before and after receiving adjuvant chemotherapy and from the healthy individuals (control group 2). The results obtained regarding estimation of TDGF-1 levels in colorectal carcinoma patients revealed that there is a higher significant difference in their concentrations before treatment (3.0 ng/ml) and after chemotherapy treatment (1.2 ng/ml) . TDGF-1 level in control group 2 was (0.1 ng/ml). that means , the expression of this TDGF-1 marker in cancerous patients was higher compared with low in normal tissues make it is the target of chemotherapy so evaluation of this marker during receiving chemotherapy give an effective results to choose the best mode of treatment and exclude the non effective ones.

Key words: *colorectal carcinoma, TDGF-1*

Introduction

Colorectal cancer is the most common cause of death from cancer after cancer of the lung and breast. In the last 30 years, there is an improvement in the treatment protocols but the incidence increased due to changing in the lifestyle [1]. No early detection of the disease associated with high mortality rate and poor prognosis. In patients with disseminated disease at the time of presentation, the median survival is 7 months the cause of death is liver metastasis [2]. Teratocarcinoma-derived growth factor 1 is a protein that in humans is encoded by the TDGF1 gene [3] The protein is an extracellular, membrane-bound signaling protein that plays an essential role in embryonic development and tumor growth. Teratocarcinoma-derived growth factor-1 (TDGF-1) is found in different body tissues. The molecular weights of TDGF-1 proteins are 24, 28, and 36 kilo Dalton ,the differences in protein sizes is related to the modifications of their structure such as addition, removal, of the groups to the core [4]. The expression of TDGF-1 is reported as a prognostic factor [5]. Colorectal cancer is aggressive cancer due to the imbalance in the signal transduction pathways such as the WNT, RAS-MAPK, PI3K and transforming growth factor-β (TGF-β) pathways. Teratocarcinoma-derived growth factor plays a key role in all of these pathways and is deeply involved in early embryo development and cancer progression [6]. High levels of *TDGF-1* mRNA and protein are expressed in a majority of human colon carcinoma cell lines and in 60–70% of human primary and metastatic colorectal tumors [7]. *TDGF-1* expressed in breast, lung, cervical, skin and ovarian cancers

Corresponding author:
Mohammed A.Hamad
dr-moh75mnr@uofallujah.edu.iq; 009647818630925
Poster address:(Zip: 00964, university of Fallujah)/ education hospital ; Fallujah, Anbar ,Iraq

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[8-10]. In normal tissues, this gene also expressed in few amount in contrast to patients with cancer of colon that characterized by high expression.

Materials and Method

Sixty two Iraqi patients (62) with colorectal carcinoma were selected in present study with age range from (8 – 47) years. The histopathological presentations of these patients were identified by the assistance of histopathologists. The patients were admitted for department of surgery in Al-Ramadi Teaching Hospital from the period (March 2013 till August 2013). The personal information for each patient was obtained. Ethical permission to conduct the research was obtained from this hospital and from all patients under study.

Control group 1 included the same patients before starting the chemotherapy (before treatment)

Control group 2 ten healthy individuals with no history of CRC, the ages and genders were matched with the studied patients .

Blood samples were taken from each patients before and after receiving adjuvant chemotherapy and from the healthy individuals (control group 2). About five ml venous blood samples were placed immediately into a plain test tube without anticoagulant, left it to clot at room temperature and then the sera of those patients were obtained by centrifugation at 2500 round per minute (r.p.m.) for 10 minutes, these sera were kept at –20°C until used for immunoassay.

Methodology

Immunological Detection of TDGF-1

To measure the concentrations of the studied marker TDGF-1 , the ELISA technique was achieved in the present study as the following:

Anti-TDGF-1 monoclonal antibodies were adsorbed to the microtiter plates and incubate for 1 h at room temperature. Two percent (2%) Bovine Serum albumin **(BSA)** was added to the plates for 1 h at room temperature . The plates were washed three times with washing buffer .One hundred µl of undiluted serum samples were added per well and incubate overnight at 4°C . Unbound TDGF-1 was removed by treating the microtiter plates with this buffer .Anti-TDGF-1 antibodies were added for one hr. at 25 C. The plates were washed with buffer and goat anti-rabbit IgG conjugated to horseradish peroxidase were added for one hr. at 25C. Plates were washed with buffer and 150 µl/well of TMB peroxidase substrate was added . Plates were incubated for a short time . Hydrochloric acid (HCl) of 1% concentraion was added for 5 minutes, then the absorbance at 450 nm by spectrophotometer was carried out.

Results and Discusion

Figure 1 shows the incidence of colorectal carcinoma with related to the patient's gender. It is obvious that males have more incidence rate than females { 41/62 (66.1%) via 21/62 (33.9%)} in respectively. Figure 2 The results obtained regarding estimation of TDGF-1 levels in colorectal carcinoma patients revealed that there is higher a significant difference in their concentrations before (3.0 ng/ml) , after chemotherapy treatment (1.2 ng/ml) and the level of TDGF-1 in the control group 2 was (0.1 ng/ml). From the present study it is clear that the expression of TDGF-1 is highly predominate in colorectal carcinoma as compared with the control group. High levels of this protein have been found in a high percentage of primary and metastatic colorectal cancers. Other methods of diagnosis of TDGF-1 indicate a variation of its expression for monitoring of the progress of the CRC, this is related to a procedure that used, for example about 68% of those patients express this marker (TDGF-1 mRNA) by using North blot technique, only 3% of patients of adjacent colon mucosa while about 75% of those patients were positive to this marker by using immunohistochemical analysis. TDGF-1 molecules can activate the signal transduction pathways of the cells and interact to the other factors such as Transforming growth factor beta1 to do its biological activity [11]. High levels of TDGF-1 expression in cancerous patients and low or absent in the tissues of the normal individuals make it a suitable marker to follow up these patients. Many strategies are used to inactivate this marker . one of them is prevent its synthesis or destruction its specific receptor. Therefore, one mode for treat those patients is synthesis of antisense therapy to reduce the *TDGF-1* gene expression by tumor cells by binding to mRNA to prevent translation process by cell ribosomes. Other strategy is by neutralization antibodies to block TDGF-1 activity. These modes are very important to impair TDGF-1 activity in management of CRC patients [12]. the best mode of treatment is achieved by combination of

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antisense therapy with TGF alpha suggesting that there are many regulator proteins that able to activate the cell proliferation and tumorogenesis of colon [13]. New mode of treatment includes combinations of TDGF-1 oligonucleotides with chemotherapy drugs in colon cancer cells. To prove this note, the treatment of tumor cells with 5FU mitomycin C and other chemotherapy induce inhibition of tumor growth before treated with antisense therapy. This is related to synergistic effect of both modes of treatment to increase the clinical outcome of patients. Anti TDGF-1 antisense therapy was developed , these contain a chemical structure that facilitate high affinity with target mRNA to increase its biological activity to block the tumor cell in vitro. In one study on immunocompromised mice, treated with different combinations resulted in inhibition of growth of colon cancer. Treatment of tumor cells with the combination of TDGF-1 antisense therapy and TGFα resulted in decreasing the levels of angiogenesis in the treated tumors (TDGF-1 with a pro-angiogenic effect). Antisense therapy used in combination with other agents to block the signal transduction pathways. The principle of action of antisense therapy that used in treatment of CA colon combine with anti-human EGFR monoclonal antibody and with is to inhibit type I protein kinase A. Metastasis from CRC adenocarcinoma can be found at the time of diagnosis in 20% of patients, and an additional 50–60% will develop metastatic disease at the time of progression. The lung is a common site of CRC metastasisTherefore, a judicious approach to diagnosis is fundamental. In our case, immunohistochemistry results combined with a previous history of rectal adenocarcinoma pointed to the diagnosis of metastatic disease. However, other clinical presentations may present a diagnostic challenge, including cases of recurrence with a single metastatic lesion or new cases of occult primary malignancies with lung metastasis. Finally, if available, concurrent evaluation of the primary tumor pathology can be of great help in achieving the correct diagnosis and, in consequence, in offering our patients the most appropriate treatment.

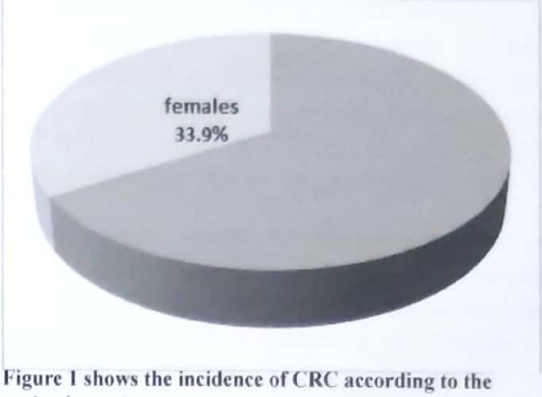

Figure 1 shows the incidence of CRC according to the patient's gender.

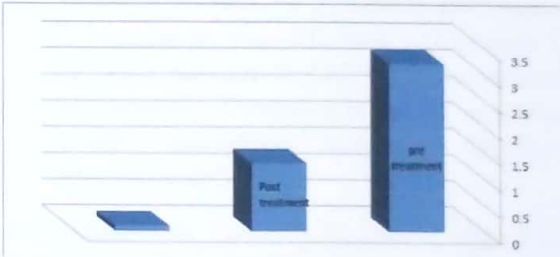

Figure 2 shows the levels of TDGF-1 before and after receiving chemotherapy

Conclusion

(ELISA) technique is very easy, simple and available method for monitoring of patients with colorectal carcinoma (CRC). TDGF-1 is a novel target marker to achieve this goal. The expression of this marker in malignant tumor and no in the normal tissues make it is the target of chemotherapy so evaluation of this marker during receiving chemotherapy give an effective results to choose the best mode of treatment and exclude the non effective ones.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Medicine/ University of Anbar and all experiments were carried out in accordance with approved guidelines.

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