ABSTRACT

The current study was focused on patients with Colorectal Cancer between the ages of 26–82 years. The objective of this study was to determine the interleukin (IL-17) level and CD33 expression status in patients with Colorectal Cancer. A total of 60 out of (40 patients and 20 control groups) were collected from gastroenterology and liver diseases teaching hospital from March 2018–May 2018, Iraq. The results show Median IL-17 was significantly higher in study group than in control group (p < 0.001), 12.13 (9.73) pg/ml versus 0.41 (0.67) pg/ml, and Median CD33 was significantly higher in study group than in control group (p < 0.001), 73.00 (5.0)% versus 4.50 (3.75) %.

Keywords: CD33, Colorectal Cancer, IL-17.

INTRODUCTION

Colorectal adenocarcinoma refers to the malignancy of the epithelial cell origin; it’s considered the most common cancer that affects the lower gastrointestinal tract (colon and rectum) and a significant contributor to morbidity and mortality worldwide. Perhaps it’s only cancer that starts as a benign adenomatous polyp; it takes years to become malignant through a sequence of genetic mutations influenced by environmental factors. Colorectal cancer incidence varies around the world. Nearly 1,200,000 new CRC cases occur globally, which represent 10% of all incident malignant tumors. In Iraq, CRC is considered the fourth most common malignancy of the lower GI that affecting both genders. Colorectal cancer comes after bronchus and lung, urinary bladder, and leukemia in the case of the male gender.

On the other hand, it comes after breast cancer, Leukemia, and brain & other CNS cancer in the case of the female gender. Based on Iraqi cancer registry 2014, it is believed that 812 (7.12%), 689 (4.86%) new cases of CRC affecting the male and female, respectively. IL-17 is an inflammatory cytokine produced by a wide variety of leukocytes, including T cells natural killer cells (NK cells), lymphoid tissue inducer-like cells (LTi-like cells), and neutrophils. Among these cells, IL-17 is reported to be predominantly produced by activated CD4+ T cells (Th17 cells). It is generally accepted that Th17 cells are induced from naive CD4+ T cells by IL-6, IL-1β, TGF-β, and IL-23, which upregulate the expression of retinoic acid receptor-related orphan receptor-γt (RORγt) via activation of signal transducer and activator of transcription-3 (Stat3) and interferon regulatory factor 4(IRF4).

MATERIAL AND METHODS

Patients and Control Groups

A case-control study has been constructed and consists of a total (40) patients, which divided into (male n = 25 and female = 15) with age ranged 26–82years. This study material was collected from gastroenterology and Liver diseases teaching hospital from March to May 2018; all patient case sheet has been recorded. Paraffin-embedded tumor tissue and normal donor colonic tissue that involves in this study were sectioned to the thickness of (3um) and spread on a positively charged slide for indirect immune-fluorescent technique. This study used the WHO grading system of colorectal carcinoma and modified Duck’s staging system of colorectal carcinoma. Five milliliters of blood were collected from the patients and healthy control then immediately transferred 2ml in to EDTA tube and 3ml into gel tube allowed to coagulate at room temperature then centrifuged at 3000 rpm for 5 minae and the serum was divided into aliquots in Eppendorf tubes until estimation and stored at (-20°C) until assay.

Indirect immunofluorescence technique for detection of CD 33 positive myeloid-derived suppressor cells on formalin-fixed paraffin-embedded tissue FFPE.

Preparation of tissue section and reagents

- Paraffin embedded tissue was sectioned to the thickness
of 3–4 micrometers, placed on positive charge slide, and left overnight at room temperature to dry.

- A 50 mL of 20X concentrated detergent wash buffer was diluted into 1000 ml of distilled water.
- The primary antibody was diluted to 1:100 for CD33 monoclonal Ab.
- Secondary antibody FITC labeled was diluted to 1:100.
- Absolute ethanol was diluted in distilled water to 95%, 70%, and 30%.

Indirect immunofluorescence procedure

- Dewax the paraffin-embedded tissue section by placing the slide in a hot air oven at 70 c for one hour, then immersed the slide in xylene, alcohol and distilled water containing jars as the following:
  - Xylene for 5 min
  - 95 % ethanol alcohol for 5 minutes
  - 70 % ethanol alcohol for 5 minutes
  - 30% ethanol alcohol for 5 minutes
  - Distilled water for 5 minutes
- The slide then tipped over a tissue paper to remove the ruminant distilled water.
- Pin pen was used to circle the tissue to prevent the diluted antibody not to spill out the slide.
- The humid chamber was prepared, and the slide placed in it, the diluted primary antibody was added, than incubated at 37c for one hour.
- After the first incubation, the slide was washed with phosphate buffer saline and tipped over the tissue, then secondary antibody was added and incubated at 37c for one hour (this step was performed in darkfield).
- After the second incubation, the slide was washed with phosphate buffer saline and tipped over highly absorbable tissue paper to remove the ruminant antibody (this step was performed in darkfield).
- Two drops of aqueous mounting media were added to the slide and covered with a coverslip.
- The slide was stored at 4 c overnight.
- The CD33 positive MDSCs were visualized under the fluorescent microscope in the darkroom.

MEASUREMENTS

The IL-17A Elisa MAX Deluxe Set. Concentration was measured that was supplied by BioLegend, U.S.A) according to the manufacturer’s instructions.

STATISTICAL ANALYSES

The SPSS software (version 23) as used for statistical analysis. The difference in gender, smoking status, alcohol status, and mutational group were examined by Pearson chi-square numerical data were presented as mean ± SD. One way ANOVA test was used to compare the tumor differentiation groups and mutational status. A p-value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

The variable IL-17 was not normally distributed according to the Kolmogorov-Smirnov test, and hence median and inter-quartile range were used to describe central tendency and dispersion instead of mean and standard deviation. Median IL-17 was significantly higher in study group than in control group (p < 0.001), 12.13 (9.73) pg/mL versus 0.41 (0.67) pg/mL, respectively, as shown in Figure 1.

The variable CD33 was not normally distributed according to the Kolmogorov-Smirnov test, and hence, median and inter-quartile range were used to describe central tendency and dispersion instead of mean and standard deviation. Median CD33 was significantly higher in study group than in control group (p < 0.001), 73.00 (5.0) % versus 4.50 (3.75) %, respectively, as shown in Figure 2.

The present study indicated that IL-17 could have a tumor-promoting activity. Moreover, the majority of studies consider that IL-17 acts as a promoter in tumor initiation and progression. Particularly, the ablation of IL-17A can inhibit the progression of spontaneous intestinal tumorigenesis in ApcMin/+ mice. Following studies in other cancers, growing evidence has shown that IL-17 can also promote tumor progression in CRC. In the intestinal tumor-bearing model, the tumor size is significantly reduced in IL-17 gene-knockout mice compared with wide-type (WT) mice, and anti-IL-17A monoclonal antibody treatment results in decreased tumor size in the WT mice. In vitro, IL-17 and TNF-α synergistically promote carcinogenesis by stimulating glycolysis and growth factor production by CRC cells. In colitis-associated cancer model, tumorigenesis and inflammatory cytokines, including IL-6, IFN-γ, and TNF-α are markedly decreased in IL-17-deficient
Correlation between Log IL-17 and Log CD33. 

Figure 3: Correlation between Log IL-17 and Log CD33.

mice compared with WT mice, suggesting that IL-7 plays a pivotal role in promoting CRC initiation in colitis-associated cancer. Based on these findings, we propose that the pro-tumor activity of IL-17 in CRC microenvironment may exert in several aspects: (1) promoting tumor elicited inflammation which facilitates the proliferation and survival of malignant cells, (2) forming an immunosuppressive tumor microenvironment by chemo-attracting immunosuppressive cells and cytokines, (3) suppressing cytotoxic cell-mediated immunosurveillance against tumor, (4) fostering tumor angiogenesis to promote tumor growth and metastasis, and (5) inducing cancer-initiating cells, which facilitates tumor malignant progression and escaping from host immune surveillance. Whereas, another study has shown that adenoma-linked barrier deterioration leads to microbial products invasion and triggers IL-23/IL-17-mediated tumor growth in CPCAPC mouse model. Despite the existing controversy presumably derived from the different models, most investigators appreciate IL-17 as a promoter in CRC progression. A previous study conducted by L. I. L. et al., 2016 came in an agreement with the present study since they demonstrated that the expression of IL-17A was increased in Vδ2 T cells in colorectal cancer patients. Previous literature mentioned that tumor progression is affected by the complicated interaction of tumor cells, stromal cells, immune cells, and related cytokines in the tumor microenvironment. IL-17 produced by epithelial cells and immune cells plays an important role in CRC development. Increased IL-17 concentration is detected in the serum of CRC patients compared with healthy donors. Moreover, it is proposed that IL-17 may act as a valuable tumor marker in patients with CRC and that concomitant expression of p53 and VEGF may provide further information about tumor features.

Furthermore, elevated Th17 cells have been observed in more than 80% of human sporadic colon cancer tissues, indicating that IL-17 expression may be one of the potential biomarkers for the future development of a new prognostic “test set” for sporadic CRC. Univariate and multivariate analysis reveal that 5-year survival rate is 72.41% in the 26 cases with lower IL-17 expression and 38.08% in the 26 cases with higher IL-17 expression, proposing that IL-17 is an independent prognostic factor for overall survival, and IL-17 producing cells may facilitate development of CRC by fostering angiogenesis via stimulation of VEGF production by cancer cells. The present study showed that the CD33+ was present at a very low proportion in the colonic tissue healthy population. CD33+ MDSCs are identified as a population of myeloid cells at earlier stages of differentiation. Because both IL-17 and CD33 were not normally distributed, log transformation was used to normalize their distribution to carry out the Pearson correlation, and the result was shown in Figure 3. Although the correlation between log IL-17 and log CD33 was positive (r = 0.284), in statistical terms, it was not significant (p = 0.075). However, this value of 0.075 is not so far from 0.05, and an increasing sample size may produce a significant correlation.

The explanation for such a positive correlation between IL-17 and CD33+ cells (MDSCs), could be explained according to the studies that they demonstrate that innate γδT cells are the major source of IL-17 in human CRC. A γδT17 cell activation is triggered by IL-23, which is highly expressed in human CRC tissues. The source of IL-23 is mainly from tumor-infiltrating inf-DCs, which is activated by microbial pathogen invasion as a consequence of tumorous epithelial barrier deterioration. These γδT17 cells secrete not only large amounts of IL-17 but also other cytokines, including IL-8, GM-CSF, and TNF-α. More importantly, the in vitro studies demonstrate that tumor-infiltrating γδT17 cells chemo attract PMN-MDSCs and further expand and provide a survival advantage for them to maintain immune-suppressive activity via secretion of these cytokines. Also, they demonstrate a strong and positive correlation between tumor-infiltrating γδT17 cells and advanced clinicopathological features, including TNM stages, tumor sizes, and lymphatic and vascular invasions of poor clinical outcome. By taken together, these findings suggest that innate γδT17 cells contribute to human CRC development and progression.

REFERENCES

dedifferentiation and acquisition of stem-cell-like properties.”


