Prognostic evaluation of Interleukin 17A and Myeloid Derived Suppressor Cells in Colorectal cancer patients.

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Abstract

The current study was focused on patients with Colorectal Cancer between the ages of (26–82years). The objective of this study to determine the IL-17 level and CD33 expression status in patients with Colorectal Cancer. A total 60 of (40 patients and 20 control groups) were collected from Gastroenterology and Liver diseases teaching hospital from March 2018 to the end of 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) %.

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Keywords: Colorectal Cancer, IL 17, CD33.

Introduction

Colorectal adenocarcinoma refer to the malignancy of the epithelial cell origin, it's considered the most common cancer that affecting the lower gastrointestinal tract (colon and rectum) and a major contributor to morbidity and mortality worldwide, perhaps it’s the only cancer that start as 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 (1). In Iraq, CRC is considered the fourth most common malignancy of the lower GI that affecting the both genders. Colorectal cancer comes after bronchus and lung, urinary bladder and leukemia in case of male gander, on the other hand, it comes after breast cancer, Leukemia and brain & other CNS cancer in case of 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(2). Among these cells IL-17 is reported to be predominantly produced by activated CD4+ T cells (Th17 cells). It is generally accepted thatTh17 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) (3).

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 were collected from Gastroenterology and Liver diseases teaching hospital from March 2018 to the end of May 2018, all patient case sheet have been recorded .Paraffin embedded tumour tissue and normal donor colonic tissue that involve in this study were sectioned to thickness of (3um) and spread on 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 milliliter 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 centrifuge at 3000 rpm for 5 mina nd the serum was divided into aliquots in eppendorf tubes until estimation and stored at (-20°C) until assay.

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

A- Preparation of tissue section and reagents

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

2- fifty ml of 20X concentrated detergent wash buffer were diluted into 1000 ml of distilled water.
3- Primary antibody was diluted to 1: 100 for CD33 monoclonal Ab.

4- Secondary antibody FITC labelled was diluted to 1:100.
5- Absolute ethanol was diluted in distilled water to 95%, 70% and 30%.

B -Indirect immunofluorescence procedure

1- Dewax the paraffin embedded tissue section by placing the slide in hot air oven at 70 c for one hour, then immersed the slide in xylene, alcohol and distilled water containing jars as the following
A- Xylene for 5 min
B- 95 % ethanol alcohol for 5 min
C- 70 % ethanol alcohol for 5 min
D- 30% ethanol alcohol for 5min

E- Distilled water for 5 min
2- The slide then tipped over a tissue paper to remove the ruminant distilled water.
3- Pin pen was used to circle the tissue in order to prevent the diluted antibody not to spill out the slide.
4- Humid chamber was prepared and the slide placed in it, the diluted primary antibody was added, than incubated at 37c for one hour.
5- After first incubation, the slide was washed with phosphate buffer saline and tipped over tissue, then secondary antibody was added and incubated at 37c for one hour (this step was performed in dark field).
6- After 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 dark field).
7- two drops of aqueous mounting media were added to the slide and covered with coverslip.
8- The slide was stored at 4 c overnight.
9- The CD33 positive MDSCs were visualised under the fluorescent microscope in dark room.

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 of 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 between the tumor differentiation groups and mutational status. P value less than 0.05 were considered significant.

Results and Discussion

The variable IL-17 was not normally distributed according to 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.

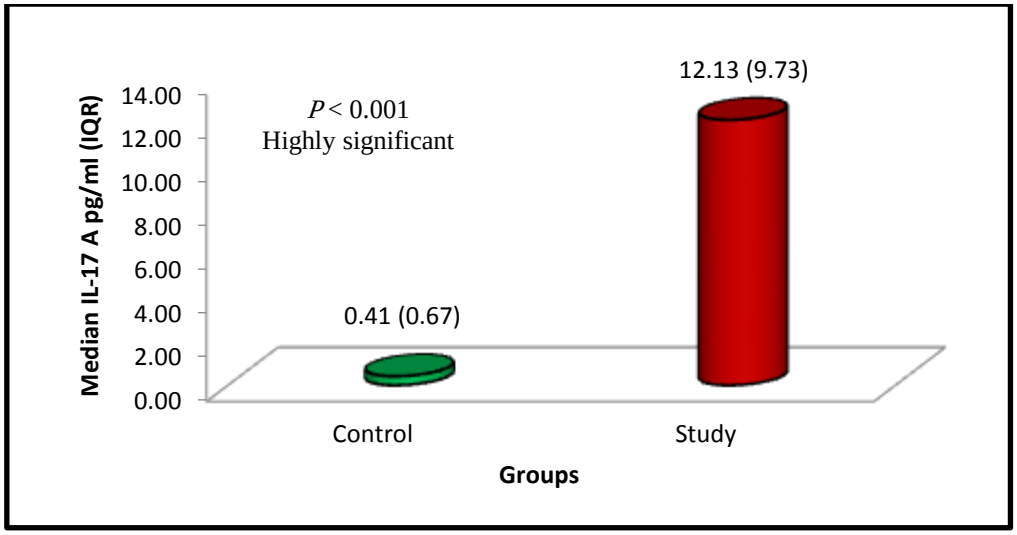

Figure 1: Median IL-17 level in control and study groups.

The variable CD33 was not normally distributed according to 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.

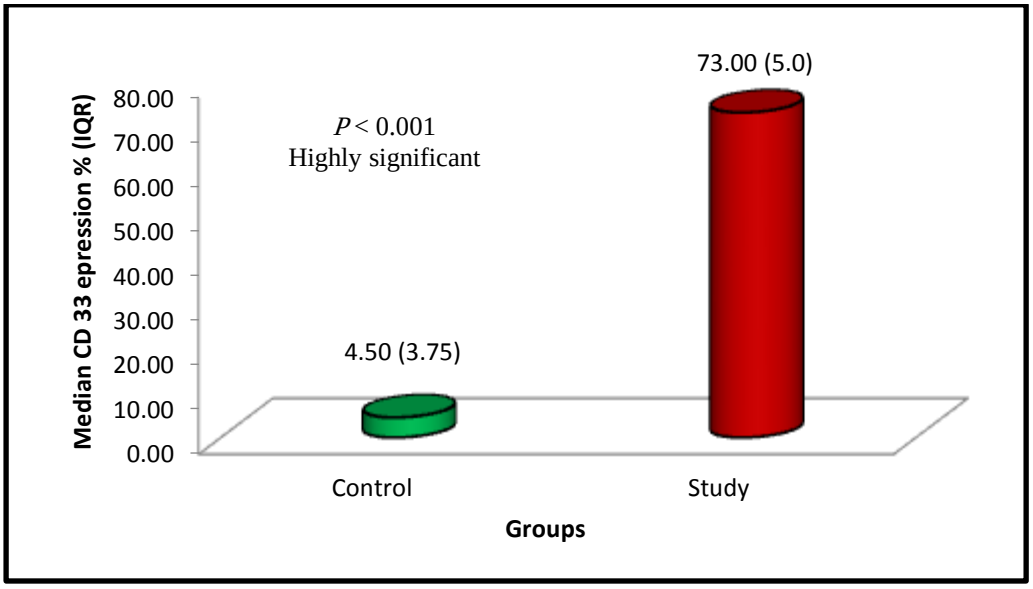

Figure 2: Median CD33 expression in control and study groups.

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 (4). In accordance with

studies in other cancers, growing evidence has shown that IL-17 can also promote tumor progression in CRC. In 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 (6). In vitro, IL-17 and TNF-α synergistically promote carcinogenesis by stimulating glycolysis and growth factor production by CRC cells (6). In colitis-associated cancer model, tumorigenesis and inflammatory cytokines including IL-6, IFN-γ, and TNF-α are markedly decreased in IL-17-deficient mice compared with WT mice, suggesting that IL-7 plays a pivotal role in promoting CRC initiation in colitis-associated cancer (7). Associated cancer (7). 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 cells-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 (8). 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. LI., et al.2016 came in an agreement with present study since they demonstrated that the expression of IL-17A was increased in Vδ2 T cells in colorectal cancer patients. A previous literatures mentioned that Tumor progression is affected by the complicated interaction of tumor cells, stromal cells, immune cells, and related cytokines in 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 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 (9). 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 potential biomarkers for the future development of a new prognostic “test set” for sporadic CRC (10). 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 (11). 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 (12,13). Because both IL-17 and CD33 were not normally distributed, log transformation was used to normalize their distribution in order to carry out 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 increasing sample size may produce significant correlation. The explanation for such positive correlation between IL-17 and CD33 + cells (MDSCs), could be explained according to the studies that they clearly demonstrate that innate γδT cells are the major source of IL-17 in human CRC. 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 not only secrete 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 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. Taken together, these findings suggest that innate γδT17 cells contribute to human CRC development and progression.

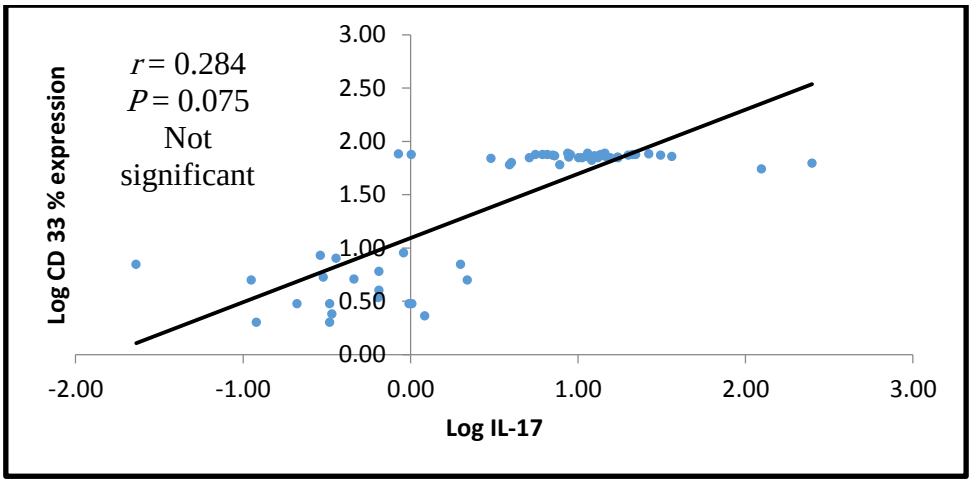

Figure 3: Correlation between Log IL-17 and Log CD33.

References:

1-Vincent T. DeVita Jr. MD, Steven A. et al.DeVita, Hellman, and Rosenberg's .Cancer: Principles & Practice of Oncology 2018

2-Cua, D. J. and C. M. Tato (2010). "Innate IL-17-producing cells: the sentinels of the immune system." Nature Reviews Immunology 10(7): 479.

3-Huber, M., A. Brüstle, K. Reinhard, A. Guralnik, G. Walter, A. Mahiny, E. von Löw and M. Lohoff (2008). "IRF4 is essential for IL-21-mediated induction,

amplification, and stabilization of the Th17 phenotype." Proceedings of the National Academy of Sciences 105(52): 20846-20851.

4-Chae, W.-J., T. F. Gibson, D. Zelterman, L. Hao, O. Henegariu and A. L. Bothwell (2010). "Ablation of IL-17A abrogates progression of spontaneous intestinal tumorigenesis." Proceedings of the National Academy of Sciences 107(12): 5540-5544.

5-Oshiro, K., H. Kohama, M. Umemura, C. Uyttenhove, K. Inagaki-Ohara, T. Arakawa, M. Harada, S. Nakae, Y. Iwakura and T. Nishimaki (2012). "Interleukin-17A is involved in enhancement of tumor progression in murine intestine." Immunobiology 217(1): 54-60.

6-Schwitalla, S., A. A. Fingerle, P. Cammareri, T. Nebelsiek, S. I. Göktuna, P. K. Ziegler, O. Canli, J. Heijmans, D. J. Huels and G. Moreaux (2013). "Intestinal tumorigenesis initiated by dedifferentiation and acquisition of stem-cell-like properties." Cell 152(1): 25-38.

7-Hyun, Y. S., D. S. Han, A. R. Lee, C. S. Eun, J. Youn and H.-Y. Kim (2012). "Role of IL-17A in the development of colitis-associated cancer." Carcinogenesis 33(4): 931-936.

8-Grivennikov, S. I., K. Wang, D. Mucida, C. A. Stewart, B. Schnabl, D. Jauch, K. Taniguchi, G.-Y. Yu, C. H. Österreicher and K. E. Hung (2012). "Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth." Nature 491(7423): 254.

9-Radosavljevic, G., B. Ljujic, I. Jovanovic, Z. Srzentic, S. Pavlovic, N. Zdravkovic, M. Milovanovic, D. Bankovic, M. Knezevic and L. Acimovic (2010). "Interleukin-17 may be a valuable serum tumor marker in patients with colorectal carcinoma." Neoplasma 57(2): 135.

10-Le Gouvello, S., S. Bastuji-Garin, N. Aloulou, H. Mansour, M.-T. Chaumette, F. Berrehar, A. Seikour, A. Charachon, M. Karoui and K. Leroy (2008). "High prevalence of Foxp3 and IL17 in MMR-proficient colorectal carcinomas." Gut 57(6): 772-779.

11-Liu, J., Y. Duan, X. Cheng, X. Chen, W. Xie, H. Long, Z. Lin and B. Zhu (2011). "IL-17 is associated with poor prognosis and promotes angiogenesis via stimulating VEGF production of cancer cells in colorectal carcinoma." Biochemical and biophysical research communications 407(2): 348-354.

12-Kusmartsev, S. and D. I. Gabrilovich (2006). "Role of immature myeloid cells in mechanisms of immune evasion in cancer." Cancer Immunology, Immunotherapy 55(3): 237-245.

13-Sica, A. and V. Bronte (2007). "Altered macrophage differentiation and immune dysfunction in tumor development." The Journal of clinical investigation 117(5): 1155-1166.