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Effect of *IL-17* Single Nucleotide Polymorphisms Gene on the Risk of Developing Colorectal Cancer in Iraqi Patients

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Abstract: Interleukin -17 pathway plays an important role in development of inflammatory bowel diseases (IBD). Moreover it has been shown that this interleukin involved in anti-tumor or pro-tumor of colorectal cancer (CRC). This study determined (70) patients male to female (60% - % 40) inflicted with colorectal cancer, range aged (<16-80) years which were collected from Baghdad Teaching Hospitals/ Medical City. The patients detected for anti – IL17 by enzyme linked immune sorbent assay (ELISA) and compared with (10) individuals as a healthy control. The results showed significant differences ($p<0.05$). The gene encoding for the cytokine IL17 has genetically polymorphic, which has (30%) single nucleotide polymorphisms (SNPs) (12out of 40) patients by single strand conformation polymorphism technique (SSCP). In conclusion, polymorphic gene *IL17* has been revealed to be associated with its susceptibility of colorectal cancer by showing there was a relationship between polymorphic gene *IL17* and the occurrence of colorectal cancer as well as the clinical features of the disease especially in aged patients.

Key words: Immunological effect IL17, polymorphic single nucleotide gene *IL17* (SSCP) and colorectal cancer.

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Introduction

Interleukin-17 (IL-17) is a novel family of pro inflammatory cytokines that consists of six similar members, designated IL-17A (also previously known as IL-17), IL-17B, IL-17C, IL-17D, IL-17E and IL-17F, according to the order in which they were discovered (1). Interleukin-17A and IL-17F lie immediately adjacent to one another on human chromosome 6, and both cytokines are produced by inflammatory T-helper 17 cells (Th17) in response to IL-23 (2,3). Several studies have found over expressing of IL-17A in various tumor tissues, including multiple myeloma, ovarian cancers, gastric cancer and breast cancer (4,5).

The hallmark of the Th17 subset is the production of interleukin IL17A and IL-17F, which share strong homology, and surface expression of the IL-23 receptor (IL-23R) (6). Interleukin-23 is essential for the differentiation of Th17 cells and plays a key role in the development of

pathogenic Th17 cells that produce the cytokine IL-17 which induces the production of several pro-inflammatory cytokines such as TNF-a and IL-6 and other chemokines (7,8,9). IL17A, IL17F and IL23R polymorphisms can affect the susceptibility to colorectal cancer. The IL17A polymorphism was positively correlated with an increased risk of developing chronic inflammation and colorectal cancer. In addition, that IL17F and IL23R polymorphisms were positively associated with colon and breast tissue mostly associated to location of the tumor (10,11). The aim of this study represented by an association between the molecular and immunological effect of cytokine IL17 and colorectal cancer.

Materials and Methods

This study utilized of 5 ml peripheral venous blood of samples which were obtained from (70) patients whom attended to Gastrointestinal endoscopy and underwent surgical resections for colorectal adenocarcinoma which diagnosed at the departments of surgery of Baghdad Teaching Hospital of Medical city. The patients proved for histo-pathologically diagnosed with colorectal cancer by medical consultants which the ratio of patients male to female was (42/28) their age (<16-80) years compared with (10) individual (male and female) as healthy control which were selected by suspected volunteer of blood donor from colonoscopy units. Blood samples were taken from patients and healthy and the sera were separated from 3 ml of blood through processing of centrifuge for 5000 RPM for 10 minutes and stored at –20 Cº until IL-17 test was measured by ELISA technique. A standard curve is prepared from standard dilutions and cytokine samples concentration is determined from a curve fitting equation as mentioned by leaflet kit (Pepro-Tech leaflet kit).

PCR Amplification

Genotype analysis for DNA which was extracted from 2 ml of the rest of venous blood of all samples using blood DNA extraction kit (Genaid / Korea) as mentioned in leaflet kit. Genotyping of SNPs within the IL-17 gene were detected by performing PCR-SSCP analysis. The primers used for SNPs represented by Forward (5′-CTGTTTCCATCCGTGCAGGTC-3′) and Reverse, (5′-TGGTGACTGTTGGCTGCACCT-3′) according to the manufacturer's instructions (Bioneer AccuPower/Korea). The amplifying of cycling program involved preliminary denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 62°C for 30 sec, 72°C for 30 sec and a final extension at 72°C for 10 min. The PCR products were run on a 1.5% agarose gel stained by Ethidium Bromide (12)

SSCP Analysis and Sequencing

Prior to loading on Non-denaturating polyacrylamide gel, PCR products were heated to 95°C for 8 minutes to denature the amplicons and then applied on ice. Electrophoresis was performed at 30 mA and 175 V for 4 h at room temperature. The ratio of acrylamide: bisacrylamide in Non-denaturing polyacrylamide gel containing 10% glycerol was 37.5:1. The DNAs electrophoresis on the gel were detected by ethedium bromide staining. The

nucleotide sequences of the PCR products that showed an abnormal electrophoretic mobility on SSCP gel were determined using direct sequencing (12,13).

Results and Discussion

The results of seventy patients confirmed with CRC percent of male to female (60% to 40%) in tested with cytokine IL-17 by ELISA technique, the study showed significant differences ($p \leq 0.05$) between patients of cancer samples and compared with normal control as mentioned in (Table-1).

Table – 1: A comparison between control and patients as related with serum IL-17 concentrations

Group	No.	Mean (IU/ml)	SE	Min.	Max.	P-value	T-test
Patients	70	379.85	90.25	7.81	3890.1	0.0119	278.03 **
Control	10	2.052	0.207	1.25	3.40		
** (P<0.01).							

The study showed there were no significant differences in both of age and gender of all the patients compare with control group as mentioned in (Tables 2 and 3).

Table-2: Serum IL-17 concentrations according to three age groups

Age (year)	No.	Mean (IU/ml)	SE	Min.	Max.	P-value	LSD-value
Less than 30	15	264.83	122.09	24.42	1596.6	0.465	409.18 NS
30-40	9	110.76	42.23	11.90	313.58		
More than 40	46	447.43	121.29	7.81	3890.10		
NS: Non-significant.							

Table-3: Serum IL-17 concentrations according to the gender

Gender	No.	Mean (IU/ml)	SE	Min.	Max.	P-value	T-test
Male	42	370.25	107.16	7.81	3167.7	0.896	270.25 NS
Female	28	394.27	160.85	8.55	3890.1		
NS: Non-significant.							

Cytokines are parts of the extracellular signaling network that controls every function of the innate and specific immune responses by operating of Th17 cells which were described as CD4 + cells secreting IL-17 (14). Several studies have shown that pro-tumor and / or anti-tumor functions of IL-17 and IL-23 (15,16) represented cytokines that necessarily maintain the Th17 phenotype through its receptor IL23R which IL-17 and IL-23 are involved in the pathogenesis of many chronic inflammatory and cancer diseases (17-20). The current study observed that this cytokine is considered as being an important mediator in an inflammation-associated of several stages of colorectal cancer of sporadic tumors of patients which were classified according to Dukes' classification system; stage A (n=8), stage B (n=29), stage C (n=27) and stage D (n=6), the tumors were localized in the colon (n= 43) and rectum (n=27) which denoted significant differences ($p \leq 0.05$) and compatibility with other studies (21-24).

Interleukin -17 induces the recruitment of immune cells in peripheral tissues. This response requires the activation of NF-kB after the commitment of IL17 to its receptor IL-17R. Also leads to the induction of many pro-inflammatory factors, including TNF-α, IL-6 and IL1 beta (25). Elevation of pro-inflammatory cytokines, such as IL-1, and IL-6 is considered to be associated with colorectal cancer (26, 27). *In vitro* assay, IL-17 induces IL-1 from macrophages and has been shown to stimulate epithelial and fibroblastic cells to secrete IL-6 (28, 29). There are two forms of IL-17; the IL-17A and IL-17F that act through a complex of two chains of IL-17RA and IL-17RCreceptor, IL-17A had significantly increased peripheral blood and tissues levels from a variety of cancer patients. In contrast, IL17F was down-regulated in human colonic cancer tissues. In this study we proved and evaluated IL-17gene single nucleotide polymorphisms which were associated with specific tumor of CRC in Iraqi populations through the results by single strand conformation polymorphisms (SSCP) technique for genomic blood extracted DNA, a product primers with M.W. (188) bps of 40 patients for detection mutations of IL-17 gene, which believed to be a critical for receptor interaction as shown in (Figures-1).

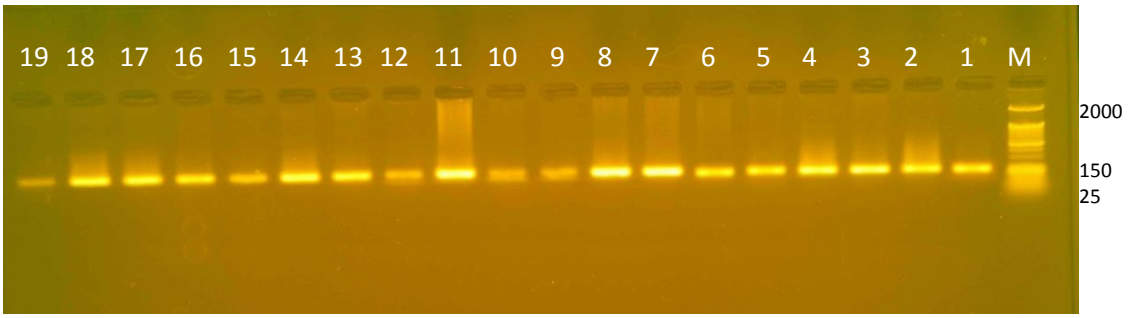

Figure-1:Gel electrophoresis of amplified *IL-17* gene, of healthy control and patients with CRC represented by samples of (No. 1 and 2-19) respectively. Bands were fractionated by electrophoresis on a 1.2 % agarose gel (2hrs., 5V/cm, 0.5X TBE buffer) and visualized under U.V. light after staining with ethidium bromide dye. (Lane M: 25bp/ Bioneer ladder)

Polymorphisms in IL-17 cytokines alter the activity of interleukins and may alter cytokine function, thus deregulating IL-17 expression (12). Furthermore, genetic studies have revealed the presence of polymorphisms in the genes *IL-17A / F* and *IL-23R* which are associated with inflammatory bowel disease and some cancers such as bladder, breast, uterus and gastric cancer (19, 30-37). Through of this study have showed that unlike IL-17 polymorphisms associated with its susceptibility to colorectal cancer which revealed by 30% (12out of 40) patients, indeed the mutated allele of polymorphism increases the risk of colorectal cancer (38-40). as shown in (Figure-2).

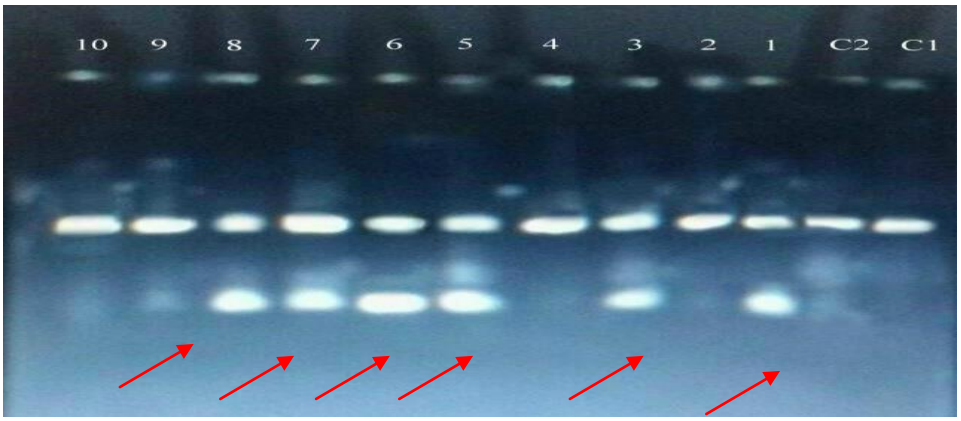

Figure-2: Bands were fractionated by vertical electrophoresis on a 40 % acryl amide gel (5 hr, 175V/cm, 1X TBE buffer) and visualized under U.V. light after staining with ethidium bromide dye. Lane: 1 (M:25bp Bioneer ladder), samples from No. (1-10) represent patients with CRC which samples (1,3,5,6,7,8) showed single nucleotide polymorphism compared with C1, C2 as healthy control.

In addition, the results of the study showed that polymorphisms of this gene are associated with clinical data and the disease severity. It has already been reported that IL-17 stimulating cells can facilitate the development of colorectal carcinoma by promoting angiogenesis, the production of VEGF, and by tumor cells. Moreover, the modulation response of IL-17 may inhibit tumor angiogenesis and enhance the inflammatory response of the host to tumor genesis (41,42). For this purpose, the IL-17A has been suggested as a new prognostic indicator in patients with colorectal cancer and could be considered as a new therapeutic target for colorectal cancer. Further a Kawaguchi and his colleagues, (1) revealed that the expression and/or activity of IL-17F may be suppressed in IL17F rs763780 polymorphism and this variant is able to block IL-8 induced by wild-type IL-17F. Besides, they suggested that the IL-17F rs763780 variant is a natural antagonist for the wild-type IL-17F and may be a potential therapeutic target (1).

Conclusion

In conclusion, the present study reported that the immunological and polymorphic effect of IL-17 may be associated with the risk of developing colorectal cancer in a Iraqi population. Therefore, may be used as a diagnostic biomarker for colorectal cancer.

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