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Assessment the of Interleukin-6 Level and Gene Polymorphism (rs1800795) at the Risk for Colorectal Cancer Patients

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Abstract: During tumor development, proinflammatory cytokines have critical role. Interleukin -6 is pleotropic cytokine that play crucial role in inflammation, immune regulation and tumorigenesis by activating multiple carcinogenic pathways. This study aimed to demonstrate an associated between IL-6 polymorphism (rs1800795) and circulating level of IL-6 with colorectal cancer in Iraq patients. Fifty blood samples were collected from both colorectal cancer patients and healthy individuals. The level of IL-6 in the serum of the study groups was determined using a specific sandwich enzyme-linked immunosorbent test (ELISA). While, the (rs1800795) polymorphisms was screened by using DNA-Sequencing technology. In the present study results revealed that the body mass index and IL-6 were significant higher in patients group compared with control group, (*P-value* = 0.0371 and 0.0006, respectively). Analysis of the IL-6 -174G/C polymorphism revealed that both in the patient and control groups, persons with the G allele had considerably higher serum levels of IL-6 than those with the C allele. (25.13 ± 1.35 *versus* 19. 81 ± 0.98, P- value= 0.0093) and (16.42 ± 0.3 *versus* 11.46 ± 0.89, *P-value*= 0.0016), respectively. It was concluded IL-6 - 174G allele could be used as prognostic biomarker for colorectal cancer in Iraqi patients. Thereby, several therapeutics targeting IL-6 may be used as promising opportunity in the treatment of colorectal cancer.

Keywords: Colorectal cancer, DNA- sequencing, IL-6, polymorphisms.

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Introduction

The most prevalent cancer in the world is thought to be colorectal cancer (CRC). It ranks second among female cancers and third among male cancers in terms of prevalence. Propagation of CRC is increasing in several areas in the world, this may relate to interaction among genetic factor, lifestyle and environmental factors (1,2,3). It is widely known that chronic inflammation plays a part in the etiology of different malignancies. Chronic inflammation linked to cancer is now recognized as a key cancer mediator and is known to affect and primarily encourage tumor survival, progression, angiogenesis, invasion, and metastasis (4,5). Generally, cytokines genes that involved in regulation of inflammatory response are genetically polymorphic and different genotypes are responsible for level of protein expression.

Distribution of cytokine genes polymorphisms may vary significantly among different ethnic groups, what could eventually contribute to observe differences in disease incidences (6). IL-6 is a pleiotropic inflammatory

cytokine which plays essential role not only in immune and inflammatory response, but also the development of malignancy (7). Human IL-6 gene located on chromosome 7 p15-p21 and consists of 5 exons and 4 introns. This gene is highly polymorphic in both 3´ and 5´ flanking regions. The single nucleotide polymorphism (SNP) in promoter site at the position -174 has demonstrated to have functional effect by alter transcription factor binding site thereby the final level of interleukin- 6 released (8,9,10). This study aimed to verified from associated between IL-6 polymorphism (rs1800795) and circulating level of IL-6 with colorectal cancer in Iraq patients.

Material and methods

Patients and control groups

This study conducted during the period December 2017 to May 2018 and included fifty colorectal cancer patients (28 males and 22 females) whom attending Endoscopic Unit and Surgery in department of Gastroenterology at Al- Yarmouk teaching hospital and fifty apparently healthy individuals (31 males and 19 females).

Samples collection

Five milliliters from blood samples were collected by vein puncture from each colorectal cancer patients and apparently healthy individuals. These samples were divided into two parts, the first one in an EDTA tubes that used for molecular tests, while the second part was put in serum separator tubes (SSTs). After that, the serum separator tubes were centrifuged at 5000 rpm for five minutes; after that serum was preserved at (-20 ºC) until used for serological tests.

Interleukin-6 serum level

Quantitation of human interleukin-6 in serum was measured by used specific sandwich ELISA kit (PeproTech, UK). All procedures were done according to manufactures' instruction.

DNA extraction

Genomic DNA was isolated from blood samples of colorectal cancer patients and apparently healthy individuals by using Wizard® Genomic DNA Purification Kit (Promega, USA). The concentration and purity of gDNA was estimated by NanoDrop 2000 spectophotometer (NanoDrop, USA). The presence and integrity of gDNA was confirm by using agarose gel electrophoresis.

Polymerase chain reaction (PCR)

The target Polymorphism of IL-6 (-174 G/C, rs1800795) was detected by PCR. The reaction was carried out with total volume 25 uL containing 1 uL from each Forward Primer (5'-TGACTTCAGCTTTACTCTTTG -3') and Reverse Primer (5'-CTGATTGGAAACCTTATTAAG -3´), 12.5µl of tag green master mix (Promega, USA), 3µl of DNA and 7.5 ul of distilled water. Thermal PCR profile for (rs1800795) polymorphism was performed as described by (11). The amplicons of PCR product (198bp) long with DNA ladder (50-800 bp) was electrophorese on 2% agarose gel stained with red safe dye and visualized under UV light using ultraviolet transilluminater.

PCR purification and DNA Sequencing

Purification of PCR product was performed by using MEGA quick-spin Total fragment DNA purification Kit (Korea). The sequencing of target fragment that flanking the (-174G/C) SNP was done by genetic analyzer 3130xl (Applied Biosystem, Foster city, CA, USA) using BigDye terminator kit (Applied Biosystem, USA). The sequencing reaction was carried out

with total volume 20µl (8µl of BigDye terminator, 1µl of primer, 4µl of amplicons and 7µl of distilled water). The cycle sequencing thermal profile was as follow: (initial denaturation at 96 ºC for 1min., followed by 25 cycle of 96 ºC for 10 sec., 55 ºC for 5 sec., 60 ºC for 4 minutes). Sequencing reaction was clean up from excess BigDye terminator by MEGA quick-spin Total fragment DNA purification Kit (Korea). Finally, purified samples were desaturated at 96 ºC for 5 minutes and placed on ice for 10 minutes. Sequencing analysis v5.4 software was used to analyze the sequence data. The sequencing results was analyzed using BLAST program available on the web site of NCBI and alignment done independently on Ref-sequences of IL-6 gene (NG_011640.1).

Statistical analysis

The effect of various factors on research parameters was determined using the Statistical Analysis System-SAS (2018) application (12). In order to compare means significantly, the least significant difference (LSD) test (ANOVA) was employed. Chi-square test was utilized to statistically compare percentages (0.05 and 0.01 probability).

Results and discussion

The present study enrolled fifty colorectal cancer patients were included 56% (28/50) males and 44% (22/50) females; mean age 53.12 years; range, 24-75 years) and fifty healthy individuals were included 62% (31/50) males and 38% (19/50) females; mean age 52.33 years; range 23- 73. Table (1) revealed the distribution of parameters that were depend in this study.

Table (1): Association among age, body mass index and IL-6 level in the study groups.

Variable Mean ± SD	Patients	Control	T-test	P-value
Age (years)	53.12 ±0.75	52.33 ± 0.86	4.392 NS	0.702
BMI (kg/m^2)	26.91± 0.60	23.17± 0.20	2.066 *	0.0371
IL-6 (pg/ml)	44.94 ± 2.33	27.86 ± 1.14	5.251 **	0.0006
*** (P≤0.05), ** (P≤0.01).**				

Results shown that there was no significant difference between cancer patient and control group as related to age (*P-value* = 0.702). While, BMI analysis showed patients have higher BMI mean 26.91± 0.60 compared to control group 23.17± 0.20 with significant difference (*P-value* = 0.0371). Many studies reported that the overweight and obesity increased the risk of colorectal cancer (13,14,15,16) and this accordance with results in this study. Obesity induce a chronic inflammation therefore, it considered the major link in microenvironment of colorectal cancer. The mean of serum level was significant higher in patients compared with control group (44.94 ± 2.33 *versus* 27.86 ± 1.14, *P-value* =0.0006). Results in the present study similar to other studies that reported that the tumor tissue and serum and expression level of IL-6 increased in colorectal cancer and have been associated with increased the tumor cell proliferation, angiogenesis, invasiveness and metastasis (17,18,19). The polymorphism (rs1800795) was specified in promoter district of IL-6 gene by using DNA sequences analysis (Figure 1).

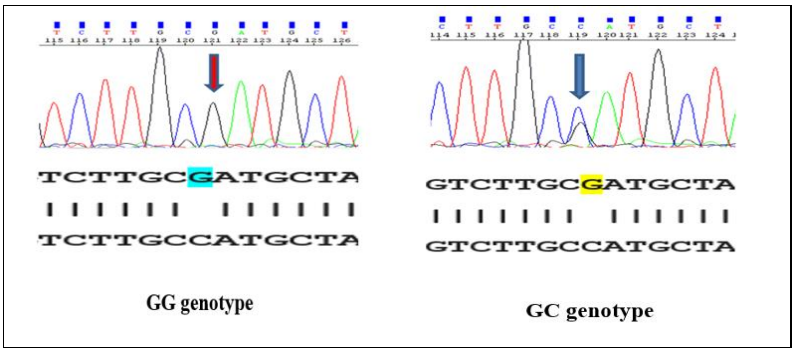

Figure (1): Electropherogram of amplicon sequencing of (rs1800795)

(Table 2) revealed that IL-6 level was significant higher in individuals whom possess GG genotype comparison with whom possess CC and GC genotypes in both patients and control groups (25.13 ± 1.35 *versus* 19. 81 ± 0.98, *P- value*= 0.0093) and (16.42 ± 0.3 *versus* 11.46 ± 0.89, *P- value*= 0.0016), respectively.

Table (2): The serum level of IL-6 (pg/ml) in the study groups according to (rs1800795) polymorphism

Groups		IL-6 -174G> C polymorphism		T-test	P-value
		Number	IL-6 pg/ml (mean ± SD)		
Patients	C−	39	25.13± 1.35	2.548 **	0.0093
	C+	11	19.81± 0.98		
Control	C−	22	16.42±0.3	2.173 **	0.0016
	C+	28	11.46± 0.89		
C−: -174 GG genotype; C+ : -174 CC and GC genotypes; ** (P≤0.01).					

Results in the present study accordance with several studies that suggested that CC genotype and C allele of – 174G/C polymorphism in IL-6 gene associated with reduce gene expression thereby IL-6 serum level and also associated with reduce colorectal cancer risk (20,21,22,23). Genetic variations may be prognostic molecular markers and polymorphisms have been considered as prospect source of cancer risk biomarkers. The polymorphism (rs1800795) that located in upstream from start site of transcription and its role with colorectal cancer has been studied in different countries; Spain (24), USA (25), Sweden (26), France (27), Canada (28) and china (29). In Iraq there was no previous studies explored impact of this SNP on predisposition of CRC risk in Iraqi population. In this study, IL-6 - 174G allele associated with increased IL-6 serum level in both study groups compared with -174C allele. Therefore, IL-6 - 174G allele may raise the risk of CRC in Iraqi patients. Inflammation is one of key factor involved in carcinogenesis. However, empirical data propose that IL-6 plays critical role not only in developed but also in the progression of metastasis from CRC. In colorectal cancer patients, high expression of IL-6 has been correlated with poor survival and IL-6 -174 genotype CC was also significantly associated with shorter survival time when compared with the heterozygous genotype CG (26,27,28,29 30). “The functional IL-6 - 174G/C SNP is situated close to a C/EBP- binding site and influence the binding of transcription factor GATA1 and the glucocorticoid receptor. As a result, this polymorphism causes reduced transactivation due to decreased ability

of the promoter to bind glucocorticoid receptor and provides promoter access to the transcriptional repressor GATA1"(31).

Conclusion

IL-6 - 174G allele could be used as predicting biomarker for CRC in Iraqi patients. Thereby, several therapeutics targeting IL-6 may be used as promising opportunity in the treatment of colorectal cancer.

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