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Association of Polymorphism GST1 Gene and Antioxidant status, and Interleukin-17 of Colorectal Cancer Iraqi Patients

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Abstract

Colorectal cancer account as the third frequent carcinoma worldwide. It is expected that most cases of CRC occur sporadically (70–80%), while around 15% of CRC case resulted from inherited factors, such as familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal carcinoma (HNPCC). A total of (60) samples collected from Gastroenterology and Liver diseases teaching hospital from April 2018 to July 2018. The aim of this present study to was to detect association of polymorphism GST1 gene (on chromosome 20) with the risk of cancer Iraq patients and compare between GST1 gene of Iraq population with gene bank of NCBI. Examine oxidative stress and antioxidant status, and Interleukin-17 of Colorectal cancer patients. A significant decrease in the IL-17A and MDA levels were showed in control compared to patients. The levels of GSH show a highly significant change in control compared to patients. The results show substitution three Transversion G>C, one Transition G>A of GST1gene and showed 99% identified with a standard in Gene Bank from patients group while having 100% identified with a standard in Gene Bank with the control group.

Key Words: *Colorectal cancer, Glutathione, Malondialdehyde, Interleukin-17A.*

Introduction

Colorectal cancer (CRC) accounts for approximately 10% of new cancers cases each year worldwide. And the odds increase with age, so screening for prevention is required for each generation. However, approximately 50% of cases of CRC worldwide located in Asia[1]. and the incidence varies among Many genes transcript for enzymes responsible for the metabolism of xenobiotics, Some of these are cytosolic glutathione S transferase (GST) enzymes which involved in phase II [2]. Those enzymes protect the important components of the cell by excluding toxic substances by catalyzing the conjugation of by-products of oxidative stress and xenobiotics to glutathione (GSH) [3]. So that antioxidant treatment may be promising protection from cancer [4]. GSH plays the main role in many metabolic processes [5,6]. Polyunsaturated fatty acids (PUFAs) oxidizes by ROS in the cell membrane. This reaction will results in lipid peroxidation, that yields free radicals [7]. IL-17 is termed as cytotoxic T-lymphocyte- associated antigen (CTLA)-8 [8,9]. It had been suggested that IL-17 act as crucial pro-inflammatory cytokine as they induce a variety of cytokines secretion by many cell types, such as mesenchymal cells and myeloid cells, that employee monocytes and neutrophils [10].

Materials and Method

Samples collection

This case-control study involves a total (60) (30 patients and 30 control groups) with age ranged (40–70years). Those samples were collected from Gastroenterology and Liver diseases teaching hospital from April 2018 to July 2018, all patient case sheet has been recorded.

A slice of paraffin-embedded tissues about of 3um placed on a positively charged slide for indirect immune-fluorescent technique. The stage and grade of these tissues classified according to the WHO grading system of colorectal carcinoma.

Five milliliters of blood was collected from each patient and control then separate 2ml into EDTA tube and 3ml into gel tube after waiting for a minute centrifuged the tube at 3000 rpm for 5 m.

Measurements

Enzyme-linked immunosorbent assay (ELISA) was used to estimate the serum level of IL-17 according to the manufacturer's instructions (BioLegend -U.S.A) and MDA was evaluated by Ghufran saad Nasif. (11), and GSH by Zayzafoon[12]. By using a spectrophotometer.

PCR Amplification

The DNA was extracted by using (DNA mini kit that was supplied by G- spin DNA extraction kit) according to the manufacturer's instructions, primer used in this study were (*GST1*) sense F: 5′-(GATTGGAATTCCGGAGGCCG-3′) and a anti sense primer R:5′-(TCTTTGGAACTCTCGCCACC-3′)' obtained from IDT company (designed) The amplification reaction conducted in volume of 25µl containing 1.5µl DNA, 5 µl Taq PCR master mix, 0.5 µl of each primer then compete for the volume to 25 µl with distilled water. Then the cycling conditions were organized as follows: Denaturation at 96 °C for 3 min, then 35 cycles of 96 °C for 40 sec, 57°C for 1.45 min and 72 °C for 45min with final incubation at 72 °C for 7 min using a thermal Cycler. After that product was electrophoresed by 1.5% agarose gel electrophoresis and visualized by exposure to ultraviolet light (302nm) after red stain staining. The sequencing of *GST1*gene was performed at Macrogen Inc., using their ABI 3730xl genetic analyzer (Applied Biosystems, US).

The statistical analysis tests were considered significant when P value <0.01, compare between control and patients group by spss program version 20 and diagram by excel version 2016.

Results and Discussion

Table 1, and figure (1) shows there was a statistically significant decrease in the IL-17A and MDA levels in control compared to patients, also the levels of GSH show a highly significant change in control compared to patients.

Table 1. The mean ± SD values of glutathione (GSH), malondialdehyde (MDA), and IL-17A in controls & patients with colorectal cancer.

Variables	State	No.	Mean	Std. Deviation	P value
MDA (µmol/L)	control	30	4.9117	1.73319	0.0001
	Patient	30	9.9600	1.72978	
IL-17A)pg/ml)	control	30	3.5286	1.78671	0.0001
	Patient	30	13.5095	6.38862	
GSH (µmol/L)	control	30	1275.9890	220.61519	0.0001
	Patient	30	665.7333	146.91610	

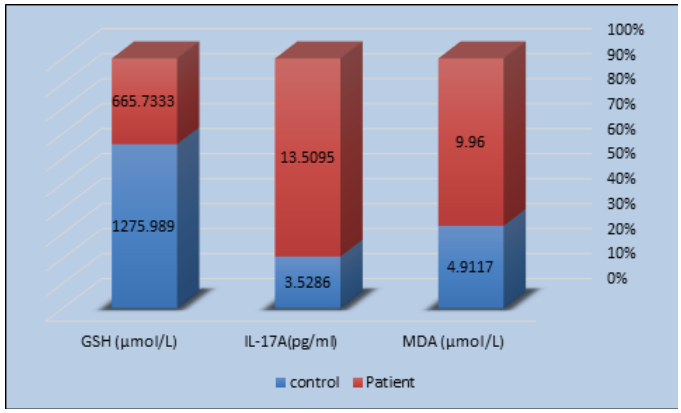

Figure (1): Levels concentration of glutathione (GSH), IL-17A, malondialdehyde (MDA) in patients and control groups.

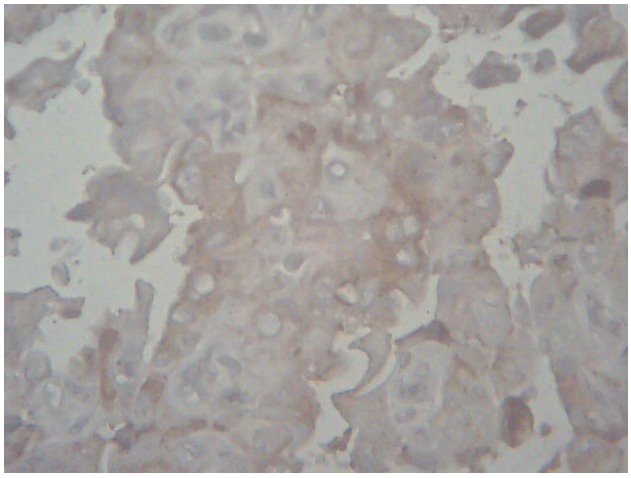

Figure (2): Immunohistochemistry of colonic adenocarcinoma.

Colorectal malignancy is the third most regular disease worldwide[13]. and the fifth most continuous kind from malignancy in Brazil[14]. Assessments for the year 2018 in Brazil are 17380 new cases for male and 18980 for female [14]. In the study by Skrzydlewska[15]. The levels of malondialdehyde in colorectal malignancy were significantly increased. This result is in accordance with previous work that announced increased MDA concentrations[16,17].This agreement with the present study. The lipid peroxidation production MDA has been reduced in patients with CRC Saintot et al. and Gerber et al. [18]. Their inference Grubben was that lower glutathione levels were associated with rising clinical hazard for the advancement from colon malignant growth[19]. Several studies showed an important lowering in glutathione levels in colorectal tumors[20,21]. In some study, Skrzydlewska et al, [22,23,24]. Indicated blended outcomes.

In CRC, the plurality from studies considers that IL-17 as a promoter in tumor initiation and development. predominately, the ablation of IL-17A can prevent the progression from spontaneous intestinal tumorigenesis [25]. The result conducted by Wu shows IL-17 is mostly considered to be a promoter in CRC progression this agreement with our study. Previous literature referenced that Tumor progression is influenced by the complexed interaction from tumor cells, stromal cells, immune cells, and related cytokines in the tumor microenvironment. IL-17 created by epithelial cells and immune cells plays a significant part in CRC evolution. Excessed IL-17 concentration is recognized in the serum of CRC patients compared with control. In addition, it is suggested that IL-17 may important as a valuable tumor marker in patients with CRC [26].

One and a half µl of genomic DNA was used for each PCR reaction. A conventional PCR protocol was utilized to analyze simultaneously the presence of *GST1* gene.

The presence of the *GST1* gene was identified by 584bp, as shown in Figure(3).

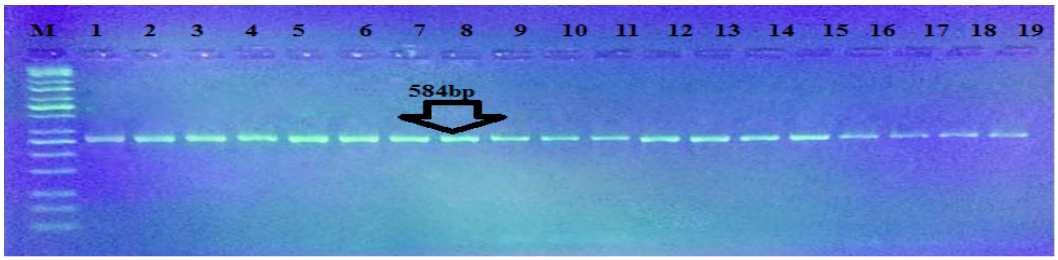

Figure (3):- Agarose gel electrophoresis for *GST1*gene (584bp). Bands were fractionated by electrophoresis on a 1.5% agarose gel (2 h., 5V/cm, 1X TBE) and visualized under U.V. light after staining with red stain. Lane: 1 (M: 100bp ladder).

The repeat of nucleotide the amplified product of *GST1*gene by direct sequencing. Our sequences were compared with the reference sequence from in national center biotechnology information(NCBI) Gene Bank.

A- G>C and G>A substitution.

After alignment of product amplification of *GST1*gene gene having three Transversion G>C, one Transition G>A from the Gene Bank, found that part of *GST1*gene having 99% compatibility with the standard in Gene Bank *GST1*gene of patients group as shown in table (2) under sequence ID: NG_008848.1.

Table (2): Represent the type of polymorphism of *GST1*gene.

Type of substitution	Location	Nucleotide	Sequence ID
Transversion	5855	G>C	ID: NG_008848.1
Transversion	5876	G>C	
Transition	5888	G>A	
Transversion	5928	G>C	

Figure (4): Alignment analysis of GST1 gene of patients group with Gene Bank of NCBI. Query represents from the sample; Subject represents a database of National Center Biotechnology Information (NCBI).

After alignment of product amplification of GST1gene for control group having no recorded change noticed in genotyping from the Gene Bank, found that part of GST1 gene having 100% compatibility with a standard in Gene Bank GST1 gene as seen in Figure (5).

Homo sapiens glutathione synthetase (GSS), RefSeqGene on chromosome 20

Sequence ID: ref|NG_008848.1|.

Score	Expect	Identities	Gaps	Strand
1493 bits(808)	0.0	808/808(100%)	0/808(0%)	Plus/Plus

Figure (5): Alignment analysis of GST1 gene of the control group with Gene Bank of NCBI. Query represents of the sample; Subject represents a database of National Center Biotechnology Information (NCBI).

Conclusions

Our study concluded in patients of carcinoma colorectal is increased as compared to healthy controls as evidenced by increased (MDA, IL-17 A) and decreased levels of (GSH) in colorectal cancer patients. GST1gene having 99% compatibility from patients group while having 100% compatibility of the control group with a standard in Gene Bank.

Conflict of Interest: There is no conflict of interest among the authors.

Funding: Self

Ethical Clearance: This study is ethically approved by the Institutional Ethical Committee.

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