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STUDY OF SEQUENCE HOMOLOGY BETWEEN MMR GENE OF *E. COLI* AND HUMAN GRNOME

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ABSTRACT

This case control study aimed to clarify the possible role of DNA mismatch repair genes MutS, MutH, MutL in commensally Escherichia coli in human colon associated with colorectal cancer through evident presence of sequence homology with human mismatch repair genes $HMlh_1$, Msh_1, Msh_2, Pms_1, Pms_2. Whole blood and biopsy samples were collected from (75) patients with colorectal cancer, and (75) other healthy person as control cases. In addition to (75) swab from colon to isolate and study the commensally E. coli form. All samples were collected from Hilla Babel province during Jan (2014) to Feb (2015). The genotyping was carried out by using PCR technique. Through studded the sequence homology between these genes showed that presence the similarity or sequence homology between muts and mutl genes of E. coli with msh_2 and $hmlh_1$ genes in human.

KEYWORDS: MMR, Colorectal Cancer, Sequence Homology

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Original Article

INTRODUCTION

Colorectal cancer is the third most common cancer. The mortality rate is also decreasing, which may reflect advances in detection and screening as well as the increasing use of combination therapies. Nevertheless, recurrence continues to be a serious problem [1]. The post replication DNA mismatch repair (MMR) system is responsible for the maintenance of DNA fidelity though replication [2]. MMR captures errors in the newly synthesized DNA strand that are missed by the polymerase proofreading and lowers the mutation frequency by a factor of (100-1000) fold as compared to MMR deficient cells [3]. *E. coli* is a gram-negative bacterium which is non-sporulation facultative anaerobe and an inhabitant of the intestines and faeces [4]. *E. coli* is found in the gut microbiota consists about of (500) species of bacteria that total (1011) cells per gram of large-intestinal content. Also other anaerobic bacteria are found in large-intestine but *E. coli* is the predominant aerobic organism in the gastrointestinal tract [5].The understanding of molecular genetic has rapidly grown over the last two decades and many genes involved in the disorders of the gastrointestinal (GI) tract such as colorectal cancer (CRC) and inflammatory bowel disease have been identified such as $hMSH_2$, $hMSH_3$, $hPMS_2$, $hPMS_1$, and $hMLH_1$ [6]. The bacteria are often overrepresented in these individuals, with *Escherichia coli* being the most prevalent species. It is clear that a complex interplay between the host, bacteria and bacterial genes is implicated in the developing or preventing of these intestinal diseases [7]. The degree of similarity between sequences of three genes (*Muts, MutH, MutL*) of mismatch repair system in *E. coli* had been studded. Studies of DNA sequence homology provide useful information about the genetic relatedness of genes, gene products, and species [8].

Patients and Methods

A total of (225) clinical samples were collected from (75) patients suffering from colorectal cancer, biopsy, blood sample, rectal swab were admitted to different hospitals, from different cities: Al-Hilla Hospital General Education, Digestive Center in Morgan City Medical, from Babylon, Al-Hussein General Hospital, from Sacramental Karbala, in addition to samples taken from private clinics during the period from (1/2014) to (3/2015).

Control

Seventy fife venous blood samples, (75) samples of tissues and (75) rectal swabs were taken from apparently healthy persons who apparently healthy included (75) persons with age range approximately matched to that of patients.

Collectopn of Specimens

The proper specimens collected for bacteriological analysis. Those specimens were collected in proper ways to avoid any possible contamination [9].

Biopsy

Different size of tissues was obtained through colonoscopy before surgery or tissue excised after surgery when diagnosed by histopathology as colorectal cancer, the textile samples preserved by formalin before use. For the purpose of extracting DNA must prepare fabric textured with liquid nitrogen (-80˚C) to break down the cells and facilitate the extraction of DNA by way of existing in the kit.

Blood

Five ml of the whole blood samples were obtained from patients in EDTA tube and kept in the refrigerator until use for the purpose of extracting DNA. Samples pass through several solutions in the comic book user then reveals DNA by electrical relay to make sure of the success of the extraction.

Rectal Swab

A sterile swab was inserted well into the anus and then placed in a cotton-plugged sterile tube for transport. The swab was inoculated on culture media and incubated aerobically at (37°C) for (24 hrs).

DNA Extraction from Gram Negative Bacteria

This method was made according to the genomic DNA purification Kit supplemented by the manufacturing company [Viogene, Taiwan].

DNA Exreaction from Blood Sampls

This method was made according to the genomic DNA purification Kit supplemented by the manufacturing company [Viogene, Taiwan].

DNA Extaction from Tissue Sample

This method was made according to the genomic DNA purification Kit supplemented by the manufacturing company [invitrogen-USA].

Detection of MMR Gene of Human by PCR

The main MMR genes were determine for all isolates by using targeting three genes, $hMLH_1$, $hPMS_1$, $hPMS_2$, $hMSH_2$, $hMSH_3$. The PCR amplification mixture has been prepared according to the manufacturer's instructions.

Detection of MMR Gene in *Escherichia Coli* by PCR

The main MMR genes were determined for all isolates by using targeting three genes, *MutS*, *MutL*, *MutH*. The PCR amplification mixture has been prepared according to the manufacturer's instructions.

RESULTS AND DISCUSSIONS

Patients

This study involved (225) patients with positive colorectal cancer (CRC) in different site of colon, included (75) biopsy, (75) blood samples and (75) rectal swabs. The study also included samples (75) blood samples, (75) biopsies and (75) rectal swab as control samples. All patients with CRC were primarily diagnosed depending on symptoms such as (change in bowel habits, general abdominal discomfort, weight loss with no apparent cause and constant tiredness) then two main strategies had been used: fecal occult blood test (FOBT) and endoscopy as primary detection of CRC. The results revealed the wide range of patients with CRC have a positive result of occult blood tests; this is going with other studies carried out by [10] and [11]. These positive results had been followed by endoscopy for confirmation. The control group consisted of (75) healthy persons with no signs of disease and normal bowel activity, fecal occult blood test (FOBT) with negative result.

Isolation of Escherichia Coli

Seventy fife rectal swab were obtained from intestinal section of (75) patients with CRC and other (75) from clinically healthy peoples (according to the contents and the mucosa of their gastrointestinal tracts did not indicate any abnormalities) for isolate commensally *E. coli* in colon. These isolates have been identified by conventional tests and analyzed by PCR approach for detection the effective genes (*MutL*, *MutH*, *MutS*) of MMR system in presence or absence of colon cancer and Prove the existence of sequence homology with human MMR genes (($hMLH_1$-$hMSH_2$-$hMSH_3$- $hPMS_1$-$hPMS_2$) that addressed by this study. The results showed that the genes of the MMR have been active in all isolates of *E. coli*. On the other hand, the study was showed that there is a similarity between the genes of bacteria and human genes work and the possibility of correction system in bacteria to make up for idled human genes. Genetic study of commensal intestinal bacterial flora of human might be important to monitor the processes of adaptation of intestinal bacteria to the host and its environment and to monitor the flow of bacterial strains between livestock production systems and the environment [12]. *MutL* homolog's bear this activity are found only in organisms rely on *MutH*-independent DNA mismatch repair system, this finding unveils yet other crucial function of the *MutL* protein at the strand discrimination step [13].

Analysis of Mismatch Repair System in *E. coli*

DNA mismatch repair enhancing genomic stability by correcting errors that have escaped from polymerase proofreading. One of the critical steps in mismatch repair is discriminate the new from the parental DNA strand as only the form needs repair [14]. In *Escherichia coli,* the latent endonuclease *MutH* carries out this function. However, most prokaryotes and all eukaryotes lack a *MutH* gene. *MutL* is a key component of this system witch mediates protein-protein

interactions through mismatch recognition, strand discrimination, and strand removal [15].

Seguence Homology

The degree of similarity between sequences, studies of DNA sequence homology provide useful information about the genetic relatedness of genes, gene products, and species. This study demonstrate a similarity between *E. coli* mismatch repair genes and human mismatch repair genes through amplifying *MutL* gene of *E. coli* in human blood and tissue samples Figure (1).

Also *MutS* gene of *E. coli* was amplifying in human blood and tissue samples Figure (2). The presence of similarity between MMR gene in human with those in bacteria may give an evidence that the proteins of both systems are also similar, and this means that the presence of bacteria in human intestine may be protective when this bacteria can release their proteins of MMR genes in vivo in which compensate the action of the MMR gene products of human when there are a defect or mutation in human MMR genes.

In fact, the mutation in MMR gens may occur at low frequencies in some human cancer, as seen in this study, but there is another problem in MMR genes is that these genes are exposed to silencing although they are intact in the genome, this genomic silencing when occurs in somatic cells will give rise to induce the cancer in these cells and this problem may occur in MMR genes particularly in CRC and other types of cancer. So, the presence of MMR genes do not mean that all these are functional because of silencing that may occur in MMR genes.

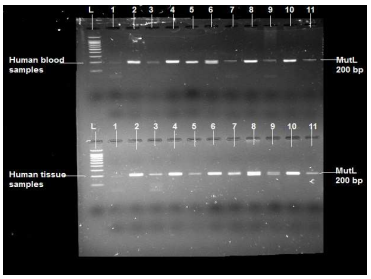

Figure 1

Figure 1: Agarose gel Electrophoresis showing functional of Mutl gene at (200) bp PCR product as a result of amplification of different human DNA samples. L(1000) bp ladder.

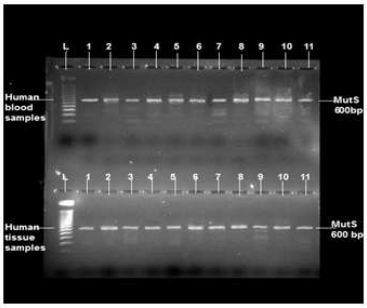

Figure 2

Figure 2: Agarose gel electrophoresis showing functional of MutS gene at (600) bp PCR product as a result of amplification of different human DNA samples. L(1000) bp ladder.

CONCLUSIONS

Cancer is commonly defined as the uncontrolled growth of abnormal cells that have accumulated enough DNA damage to be freed from the normal restraints of the cell cycle. The highly site-specific adherence of E. coli involves binding species-specific adhesions to the required cell surface receptors in colon. The role of species that colonize tumours could be causal, coincidental or potentially protective. CRC appears that colonization by commensally E. coli may reduce the risk of cancer in some populations through presence the sequence homology in some genes of MMR system in them and human MMR genes.

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