1. Introduction

Inflammatory bowel disease (IBD), including ulcerative colitis and Crohn’s disease, is on the rise across the globe (1). The IBD, especially ulcerative colitis due to its association with colonic carcinoma, is considered a global health problem (2). Although the precise etiology of IBD is unknown, a very close relationship has been observed between IBD and several factors, such as immunity (3-5), diet (6), genetics (7), and environment (8).

It has been proposed that IBD emerges when environmental factors provoke a constant, mischievous immune reaction of the intestinal mucosa towards commensal microbiota (9). The interaction of genes regulating the function of the immune system is vigorously mannered by the environment, especially gut resident microbiota and other noxious antigens (9). Toll-like receptors (TLRs) belong to the family of transmembrane proteins that recognize the microbial pattern. The TLRs are expressed in different ways either substantially or induced by several distinguishable types of cells everywhere in the gastrointestinal tract GIT (10). These proteins are conclusive entrepreneurs of innate immune responses. There are 11 well-known types of TLRs, and some of these proteins are found either on the cell membrane (TLR1, 2, 4, 5, and 9) or inside organelles (TLR3, 7, and 8) (11) and are all called transmembrane receptors.
The TLR5 is manifested in the epithelial cells of colon. The TLR5 ligand flagellin can organize the homeostasis among T effector and regulatory cells in IBD (12). Despite the importance of the aforementioned issues, little is known about the TLR-5 situation in IBD. Therefore, the present study aimed to mark the status of TLR-5 expression in the intestinal mucosa of UC patients using quantitative real-time polymerase chain reaction (qPCR).

2. Materials and Methods

This study was performed on formalin-fixed, paraffin-embedded, intestinal tissue blocks in the Department of Pathology, College of Medicine, University of Babylon, Iraq. A total of 50 samples of intestinal tissue (ulcerative colitis=25, and normal intestinal tissue= 25) with the age range of 28-60 years were included in this study. All cases were examined by two pathologists.

2.1. Extraction of RNA

TRIzol® reagent kit (Bioneer, Korea) was used to extract total ribonucleic acid (RNA) according to company instructions; thereafter, the trace amounts of genomic DNA were removed using DNase I enzyme kit.

2.2. Complementary DNA (cDNA) Synthesis

The extracted and treated 100ng/ul of RNA was reverse-transcribed for complementary DNA synthesis using the M-MLV Reverse Transcriptase kit (Bioneer, Korea). In the presence of primers, the reverse transcriptase enzyme synthesized a single-stranded DNA using an mRNA molecule as a template. The first new strand DNA molecule can then be used as a template for double-stranded DNA synthesis.

2.3. “Quantitative Real-Time Polymerase Chain Reaction

This method was used for “quantification of TLR5 mRNA transcript levels (Gene expression), while the examination of the comparative gene appearance was carried out using a method known as 2^-ΔΔCT Livak. The Real-Time PCR system (BioRad, USA) was used for qPCR reaction and by using the “SYBER Green dye qPCR master” mix used in discovery and intensification of mark genes and “GAPDH housework gene for the standardization of gene appearance. Primer sequences were as follows: a forward primer for TLR5 was 5’- TTGCTCAAAACACCTGGACAC -3’, and that of the reverse primer was 5’-TTGGCAATGCGGTTCCTCC -3’. The sequence of the primers used for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was 5’- AATTTCCATGGCACGTCGAAG -3’ (forward) and 5’-ATCGCCCCACTTGATTTTGG -3’ (reverse); (Bioneer company, Korea). The components of the PCR reactions were as follows:

“A 5 μL cDNA, 25 μL 2X green star master mix, 2 μmol/L forward primer and 2 μmol/L reverse primer 16 DEPC water were added to AccuPower™ 2XGreen Star qPCR master mix kit (Bioneer, Korea) in a total volume of 50 μL”. The magnification procedure was used as follows: initial denaturation at 50°C for 1 h and 1 cycle, denaturation for 40 cycles at 95°C for 20 sec, followed by 40 cycles of annealing at 60°C for 30 sec, and 1 cycle of melting at 60°C-95°C for 0.5 sec.

2.4. Statistical Analyses

Two software programs were used to summarize, present, and analyze the data. These programs were Microsoft Office Excel 2007 and the statistical packages for social sciences version 18 (SPSS 18) using t.test, one-way ANOVA test, Chi-square test. A p-value of ≤0.05 was considered statistically significant.

3. Results

3.1. Clinicopathological Analysis

The clinicopathological assessment of ulcerative colitis samples revealed that the majority of cases (72%) were >50. In terms of gender, 12 (48%) of subjects were males, and 13 (52%) were females (Table 1).
The fold changes in TLR5 gene expression were measured. There was a positive correlation between TLR5 fold change in ulcerative colitis and normal-looking intestinal tissue in which the mean fold change is 1.812 in ulcerative colitis, and the mean fold change is 8.251 in normal-looking intestinal tissue. This correlation is significant since the p-value is less than 0.05 (Table 2).

As illustrated in Table 3, males and females did not significantly differ in TLR5 mean fold change (P>0.05).

Regarding age, there was no significant difference between the subjects aged ≤50 and those above 50 years of age (P=9.21; Table 4).

### 4. Discussion

Intestinal epithelial cells are in direct contact with the commensal microflora. This type of interaction is known to partake in the maintenance of immunity and intestinal physiology in different methods (13). The TLRs admit varied microbe-connected molecular patterns, such as flagellin, bacterial lipoprotein lipopolysaccharide (LPS), and others which are produced by both commensal and pathogenic microorganisms (14, 15). They play a major role not only in the recognition of microbes in innate immunity but also in the activation of adaptive immune responses (16).

The disequilibrium of intestinal immune responses to microbes is thought to be the characteristic event in ulcerative colitis pathogenesis; nevertheless, the expression of TLRs in ulcerative colitis was estimated only by a few authors (17-20). The present study demonstrated the expression of TLR-5 mRNA by using qPCR and detected a decreased level of TLR-5 mRNA in the mucosa of ulcerative colitis cases, as compared to normal controls with significant difference between two levels (P=0.001; Table 2). The results of the current study are in agreement with those obtained by Stanislawowski, Wierzbicki (20) who reported decreased levels of TLR-5 protein and TLR-5 mRNA in the inflamed mucosa, as compared to normal controls.

The upregulation of TLR-5 receptors in healthy control colonic mucosa observed in this research and other studies (20) assume that under normal situations,
the tight intersection between intestinal epithelial cells prevents flagellin from evoking any significant inflammatory response. The results of this study suggested that the damaged colonic mucosa by an inflammatory process may lead to a decreased expression of TLR-5 expression, probably as a result of down-regulation caused by plenty of flagellin in the damaged epithelium undergoing the mucosal barrier.

In general, TLR5 seems to play a vital role in ulcerative colitis development and is a good target for developing an effective therapeutic strategy for the management of ulcerative colitis by stimulation with TLR-5 signaling.

Authors' Contribution
Study concept and design: A. F. A.
Acquisition of data: A. F. A. and R. G. F.
Analysis and interpretation of data: H. H. S.
Drafting of the manuscript: A. F. A.
Critical revision of the manuscript for important intellectual content: A. F. A., R. G. F. and H. H. S.
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Ethics
All instruments applied in this study were calibrated and maintained in accordance with routine quality control procedures overseen by the Quality Assurance of College of Medicine, University of Babylon, Babylon, Iraq.

Conflict of Interest
The authors declare that they have no conflict of interest.

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