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The Effect of Interleukin-37 Plasma Levels and SNP il-37 Gene in Ulcerative Colitis in Iraqi Patient's

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Abstract

Ulcerative colitis (UC) is a chronic inflammation of the gastrointestinal tract without any signs of infection. The association between genetic variations in the IL-37 gene and increase in serum IL-37 levels in UC has not been investigated. The aim of study was found the association between IL-37 genetic variants, IL-37 plasma levels, and various clinical phases of UC. Serum concentrations of IL-37 in UC reveled (56.72± 16.41) using ELISA, and genetic variants were genotyped by (PCR) and the sanger sequence technique. It was found that the G>A genotype of (rs3801147) occurs significantly more frequently in patients with active UC. Since the allele G is considered a common allele in patients, it may have a good etiological role for disease.

Keywords: IL-37, Ulcerative colitis, polymorphism, rs3801147, interleukin.

1. Introduction

In recent advanced economies, there has been a global increase in the prevalence of inflammatory bowel disease (IBD), with reports of growing incidence observed across all continents [1]. The aetiology of (IBD) patients remains unknown; nevertheless, it is hypothesized that components of the gut microbiota and other external factors contribute to the disruption of the epithelial barrier, especially in those with genetic predisposition, leads to an exaggerated immune response in the mucosal lining [2].

The complex pathogenesis of UC includes genetic susceptibility, defects in the epithelial barrier, dysregulation of immune response, microbial dysbiosis, and environmental variables [3, 4]. IBD was shown to have serological characteristics that were more like those of UC rather than Crohn's disease [5]. Ulcerative colitis (UC)

is characterized by inflammation of the colonic mucosa, which initiates in the rectum and progresses proximally in a continuous fashion, encompassing either a portion or the entirety of the colon [6].

The synthesis of IL-37 in autoimmune illnesses can be enhanced by the cytokines IL-1β and IL-10, suggesting that these cytokines contribute to the upregulation of IL-37 production [7]. The association between a particular genetic mutation in the IL37 gene, referred to as SNP rs3811047 G > A, with an elevated susceptibility to autoimmune disorders such as ankylosing spondylitis (AS), rheumatoid arthritis (RA), and stomach cardiac adenocarcinoma has been demonstrated in clinical research [8]. The aim of the study is finding relationship between IL37 gene SNP rs3811047 and concentration of IL-37serum and tTG in UC patients.

2. Methodology

The investigation was conducted in Ministry of Health and Environment of Iraq / Medical City, Baghdad Teaching Hospital, and the gastrointestinal unit at the Madinat Al-Imamain Al-Kadhimain Al-Tbbia Hospital in Baghdad between March 2022 and December 2022. The study examined a total of 180 individuals, including 90 patients severing from ulcerative colitis and 90 normal individuals.

2.1 Collection of Blood Sample

Using a disposable syringe, approximately 5 mL of venous blood were drawn from each participant (patients and controls). The blood was divided into two portions. Placed two millilitres of whole blood into EDTA tube that was kept at -20 °C until DNA extraction to determine the SNP IL-37. Other tube for blood was left at room temperature for 10 minutes to clotting, centrifuged at 6000 rpm for 15 minutes, and then serum was separated and frozen at -80 ºC until time for the ELISA test.

2.2 Determination Tissue transglutaminase and IL-37 level.

Using an tTG/IgA-IgG kit from the company Demeditec in Germany, tTG levels in the serum also, Interleukin 37 kit analysis by using the enzyme-linked immune sorbent assay (ELISA), Shanghai YL Biont, China We measured Interleukin 37 and recorded the values in tables, which we used in the statistical process to obtain the results.

2.3 Determination of SNP IL-37

2.3.1 Genomic DNA

Genomic DNA was extracted from the peripheral blood leukocytes (frozen EDTA blood samples) by ReliaPrep™ Blood gDNA Miniprep System.

2.3.2 DNA Extraction

Easy Pure ® Genomic DNA Kit use to DNA Extraction by TransGen biotech company/ China.

2.3.3 Measurement of DNA Purity and Concentration

The optical density of 1.5 µL of DNA was measured using a nanodrop UV spectrophotometer at two wavelengths (260 and 280 nm). Most of the samples had an A260/A280 ratio between 1.8 and 2.0, which is thought to be appropriate for additional analysis to identify IL-37 gene polymorphisms.

2.3.4 Agarose gel electrophoresis of DNA

After the extraction process, electrophoresis was used to identify DNA segments or to identify the outcome of the PCR interaction in the presence of a ladder to differentiate the bundle size of the PCR interaction on the Agarose gel.

2.3.5 The primers

Alpha DNA company, USA supplied the primers as shown in (table 1).

Table 1: Alpha DNA company, USA supplied the primers.

Region	SNPs	Sequence 5′…3′	Ref
Exon II	rs3801147	TCAGAAGAGGCACCTGGAAG	Alpha DNA
		AAGGGTGGGCTGAACAAATG	

2.3.6 Polymerase Chain Reaction (PCR)

The following ingredients make up the Easy Taq® PCR super Mix (+Dye). Furthermore, -20 °C is the ideal temperature for storage. DNA polymerase - dNTPs (dATP, dTTP, dCTP, and dGTP) - optimized buffer- gel loading buffer.

2.3.7 Sequencing and Alignment of Sequence

The technology developed by Sanger for gene sequencing was alternatively referred to as the dideoxy chain termination technique. The primers and PCR products utilized in this inquiry were provided to the South Korean company Macrogen (dna.macrogen.com).

2.4 Statistical Analysis

Data analysis was conducted as following: Statistical analysis: SPSS for Windows, version 22 (SPSS Inc., Chicago, Illinois, United States) was used to perform statistical analysis on the data. The data was presented in a mean, standard deviation (SD). A Shapiro–Wilk normality test was used to determine whether the studied parameters followed Gaussian distribution.

3. Result and Dissection

3.1 Tissue transglutaminase IgG/ IgA

The analysis indicates in (table 2) and results correlate with the research conducted by Bizzaro and co-workers [9], that revealed that among CD cases, 76.1 % had less than 10 anti tTG IgG antibodies, while 23.8 % had more than 10. Additionally, 85.7 % had less than 10 anti tTG IgA level, while 14.3 % had more than 10.

The production of tTg enzyme is not influenced by the patient's sex. Instead, it is released in various regions where apoptosis and tissue cell regeneration occur, depending on the extent of damage in the small intestine. In CD this enzyme is synthesized by the epithelial layer of the afflicted small intestinal tissue. Its function is to enhance the interaction between gluten protein and antigen-presenting cells (APCs) [10].

3.2 Interleukin-37 serim

The control group exhibits the highest mean IL-37 level at 91.44 ± 23.60 pg/mL, significantly higher than that of the UC groups, with mean levels of 67.42 ± 14.13 pg/mL and 56.72 ± 16.41 pg/mL. A study conducted by Li et al., [12] suggests that IL-37 has the potential to function as a biomarker for inflammatory bowel disease (IBD).

People with UC had considerably lower amounts of IL-37 in their blood compared to healthy individuals. Additionally, a negative association was observed between the levels of IL-37 and the severity of ulcerative colitis (UC). Interleukin-37 is anticipated to have a crucial role in diminishing inflammatory reactions that contribute to the onset of inflammatory bowel disease (IBD) and preserving equilibrium in colonic inflammation in humans. It is being employed as a novel cytokine therapy for inflammatory bowel disease (IBD) and other inflammatory conditions [13].

Interleukin-37 is a crucial cytokine that plays a vital role in regulating inflammation. The compound has the potential to impede the production and activity of pro-inflammatory cytokines, hence playing a crucial role in the regulation of inflammation [14]. The anti-inflammatory cytokine Interlukin-37 has been identified in various inflammatory diseases, excluding rheumatoid arthritis (RA). Furthermore, systemic lupus erythematosus (SLE) and inflammatory bowel disease (IBD) are specific medical conditions that involve inflammation of the bowels [15]. Spondylitis with ankylosing nature (AS) [16].

Table 2: Result of Tissue Transglutaminase and Interleukin-37 in UC.

Variable	*Group*	*M± SD*	*SEM*	*Mdn.*	*P value*
tTG_IgA	UC	10.46 ± 5.68	0.60	10.10	< 0.001
	Control	8.97 ± 4.99	0.53	7.95	
tTG IgG	UC	12.51 ± 6.35	0.67	12.00	0.378
	Control	11.34 ± 4.75	0.50	12.00	
IL_37	UC	56.72 ± 16.41	1.73	55.95	< 0.0001
	Control	91.44 ± 23.60	2.49	86.95	

3.2 SNP of Interleukin-37

3.2.1 The DNA extraction

DNA extraction from blood samples of patients and control groups in nanodrop and gel electrophoresis showed good purity and quantity as demonstrated in (figure 1).

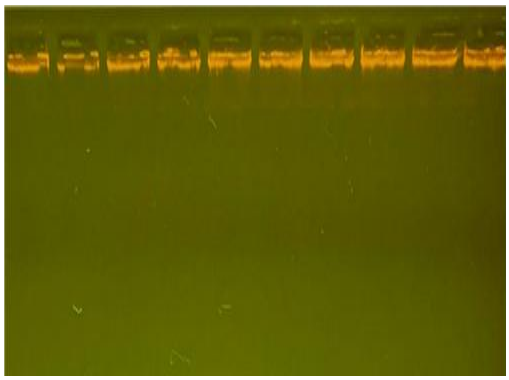

Figure 1: DNA extraction from blood samples of patients and control groups.

3.2.2 Detection of rs3811047 in groups study by conventional PCR.

After PCR reaction and gel electrophoresis samples showed has band with size 687 pb (figure 2).

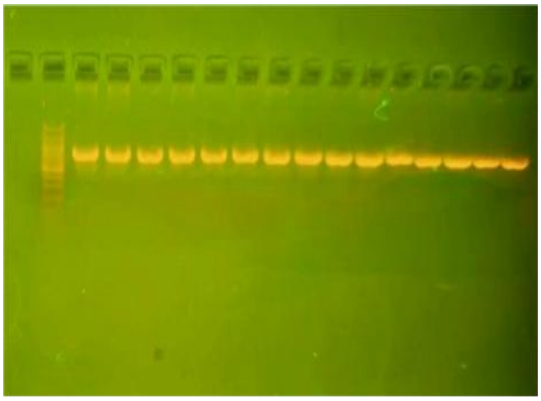

Figure 2: Samples after PCR reaction and gel electrophoresis.

The analysis of the rs3811047 genotype frequencies across UC and control groups yields significant findings in UC group (figure 3). The GG genotype is present in 58.33 % (35 individuals), the AG genotype in 16.67 % (10 individuals), and the AA genotype in 25.00 % (15 individuals).

Notably, the control group presents a different pattern, with 28.33 % (17 individuals) having the GG genotype, 16.67 % (10 individuals) the AG genotype, and a majority of 55.00 % (33 individuals) displaying the AA genotype. The Chi-square test results in a statistic of 24.748 with 4 degrees of freedom, accompanied by a highly significant p-value < 0.001.

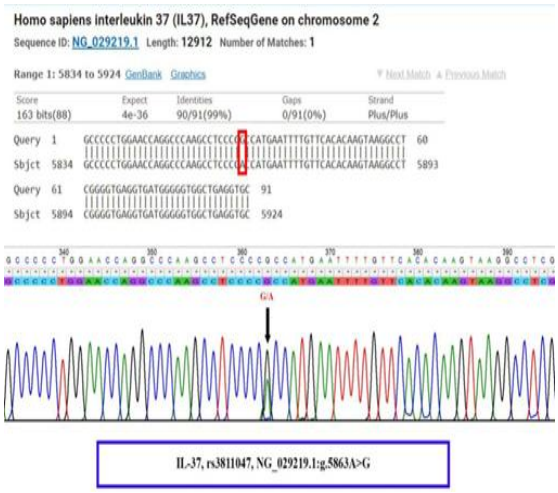

Figure 3: Analysis of the rs3811047 genotype frequencies across UC and control groups.

4. Conclusion

An established role for IL-37 in relation to IBD progression clinical state when compared to control group. This cytokine could be considered as a potential biomarker of IBD, importance of this cytokine in the pathogenesis of the disease as well as the role of genetic factor for disease susceptibility.

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