



Thiopurine Methyl Transferase (TPMT) Gene Expression in Imuran and Biological Response in Ulcerative Colitis in Iraqi Patients

¹Noor S. Ali, ¹Rafid A. Abdulkareem

¹Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad

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Abstract: Ulcerative colitis (UC) is a chronic IBD that involves the rectum and colonic mucosa. The study aim to expression in biological therapy more than its expression in Imuran treatment. This study included 50 patients with UC and 22 healthy. The RNA was extracted by the triazole method and the ages ranged from 15-50 years. The response to Imuran treatment was weak, so they were referred to biological therapy. The gene expression of 22 patients was lower than the control ratio, meaning low gene expression, and the folding percentage was over gene expression. Venous blood samples were collected and preserved in TRIzol tubes, RNA extraction was performed, cDNA was synthesized, PCR was performed, expression levels were calculated and T-tests used to measure gene expression. Thiopurines are immunomodulators used for UC, and TPMT gene expression see significant value in Iraqi patients with high expression in biological therapy more than its expression in Imuran treatment groups. It was concluded gene expression of TPMT have a significant effect in Iraqi patients with UC.

Keywords: TPMT, UC, Gene expression and RNA extraction.

Corresponding author: (Email: rashasubhi85@Gmail.com).

Introduction

Inflammation of the colonic mucosa is a hallmark of the chronic bowel disease called ulcerative colitis (UC). Rectal bleeding, diarrhea, and cramping in the abdomen are signs. In the United States alone, among 250,000 and 500,000 people have UC, and each year the rate is between 2 and 7 per 100,000 people. even though the pathophysiology of UC remains unclear, chronic inflammation is believed to be a consequence of an abnormal immune response that is brought on by both environmental and genetic factors (1).The severity of symptoms and extent of the illness serve as criteria to categorize UC

(Figure 2). a variety of factors, including the severity of the symptoms, an illness can be described as mild, moderate, severe, or fulminant (2). Endoscopy and symptom evaluation are advised by recommendations for therapy to assess the severity and extent of the disease as well as to confirm the UC evaluation (3) (4). The process of turning a gene's information into a protein is known as gene expression. Translation, mRNA splicing, transcription, and post-translational protein modification are all involved. The quantity and spatiotemporal characteristics of the functional protein profile can change depending on changes in this mechanism.

It's necessary for healthy cell development, including processes like differentiation and morphogenesis, as well as proper cell structure and function (5). While vital to normal function or survival, cells can release functional proteins by regulating gene expression. This system must exist for damage repair, homeostasis maintenance, and cellular adaptation to novel environments (6). Azathioprine (AZA) is effective for recuperation in ulcerative colitis and Chron's disease without requiring the administration of steroids, but its gradual onset may not be beneficial (7). Biologics are genetically modified illicit substances established from the by-products of organisms that block the immune response in colitis patients. In moderate to severe disease, they can be utilized to bring about and sustain remission. Adalimumab, golimumab, and infliximab all function by preventing the production of tumor necrosis factor (8). A biopsy of the colonic mucosa may reveal deformed mucosal crypt architecture and an increase in the number of inflammatory cells therein. In addition, there are numerous medication classes that can be used to treat UC. 5-aminosalicylates and sulfasalazine (8).

Thiopurine methyl transferase (TPMT) is an enzyme involved in the metabolism of thiopurines (e.g., azathiopurine) used to treat IBD. There can be a wide variation in TPMT enzyme activity that is genetically determined by the TPMT gene. According to Hayes, "normal levels of TPMT enzyme activity are found in 89% of people, 11% have intermediate activity and approximately 0.3% have little or no activity. People who have intermediate or no TPMT enzyme activity cannot undergo treatment with thiopurines (9). For prolonged periods of recuperation and steroid sparing in IBD patients, the thiopu-

rines mercaptopurine (MP) and its precursor pharmaceutical AZA are frequently employed either alone or alongside with other medications. These medications are ineffective for quick remission induction due to their slow time to clinical response (between 8 and 12 weeks). These immunosuppressants are currently utilized in biological therapies as well because they can lessen the immunogenicity of biologics (10). Thiopurines are inactive and require being switched intracellularly by a variety of enzymes into the active thioguanine nucleotides (TGN), which suppresses the immune system (11). subsequent to responding alongside thiols (such as glutathione), (MP) can be spontaneously published from AZA as well as enzymatically by glutathione transferases (12). Thiopurines are beneficial for IBD remission stimulation and maintenance. The ratio of the concentrations of me-TIMP (methyl thio-inosine monophosphate) to (6-TGN) 6-thioguanine nucleotide has been related to drug effectiveness. This study, investigated the molecular underpinnings of variations in metabolite profiles and their relationship to the progression of diseases (13).

A recombinant monoclonal antibody of the IgG1 kappa subclass with 25% murine and 75% human sequences is referred to as infliximab (IFX). The human Immunoglobulin G1 (IgG1) unchanged region and the variable murine Fab' region of infliximab are joined by bisulfide bonds (14).

The recombinant monoclonal antibody adalimumab (ADA) has govern TNF- inhibitory effects, cytotoxicity, and apoptosis. A human IgG: j constant region and human-derived variable regions constitute its basic structure (15).

Material and methods

Sample collection

Patients

There are 50 patients with chronic ulcerative colitis, males and females, from Al-Yarmouk Teaching Hospital, Al-Imamin Al-Kadhimin Medical City, and the Digestive System Hospital in the Medical City, and 22 healthy volunteers with ages ranged from 15 –50 years during the period from December 2022 to April 2023, with ages ranging from 15-50 years.

Venous blood samples (1ml) were collected from enrolled participants in sterile conditions. The remaining blood was preserved directly

in TRIzol tubes (400µl of blood with pre added 600µl of TRIzol reagent according to manufacture recommendation). (16). These tubes then were stored in freezer at -20°C until extraction of RNA for molecular study.

Molecular analysis

Primer

The mRNA sequences for the (TPMT) gene were obtained from the National Center for Biotechnology Information GenBank database. RTqPCR primers was designed using the Premier 3 software with Annealing temperatures 58C, primers lengths 24 nucleotides, and PCR amplicon lengths 172 base pairs as show in Table (1,2).

Table (1): Real-Time PCR program

Steps	°C	m: s	Cycle
RT. Enzyme Activation	37	15:00	1
Initial Denaturation	95	05:00	
Denaturation	95	00:20	50
Annealing	58	00:20	
Extension	72	00:20	

Table (2): Sequence of primer to TPMT gene and Housekeeping Primers

Primer name	Sequence	Length	Annealing Temp. (°C)	Product
TPMT	F AGCCTGAAAATGTAATGGATGAAT	24	57	172
TPMT	R TCACATCATAATCTCCTCTCCAAA	24	57	172
RPL27	F ATCGCCAAGAGATCAAAGATAA	22	57	123
RPL27	R TCTGAAGACATCCTTATTGACG	22	57	123

RNA extraction

Whole blood was withdrawn from a pipette and placed in a sterile ependron tube with triazole, chloroform, cold centrifuge, and ethanol. Washing solution 1 and 2 was used to wash the RNA. The elution process is performed by adding 75 microliters of elution buffer to each tube and centrifuged for one minute. According to the protect instructions, total RNA of UC samples was extracted by TRIzol reagent. Following the protocol of Applied Bio systems, cDNA was synthesized from 2 µg RNA using Oligo dT primers and Superscript II Reverse Transcrip-

tase (RT) transcribed in 20 µL-system using Bonier Corporation kit (Korea Bio Park BLDG) . The polymerase chain reaction (PCR) was performed using ABI prism 7900HT sequence detection system (Applied Bio systems). The primer sequences for the detection of TPMT were (forward primer) 5'AGCCTGAAAATGTAATGGATGAAT'3and (reverse primer) 3'TCACATCATAATCTCCTCTCCAAA' 5. The PCR conditions consisted of 50 cycles at 95 °C for 15 s and 58 °C for 20 s and 72c for 1min The expression level was calculated by $\Delta \Delta Ct$ method as shown in (Figure 1).

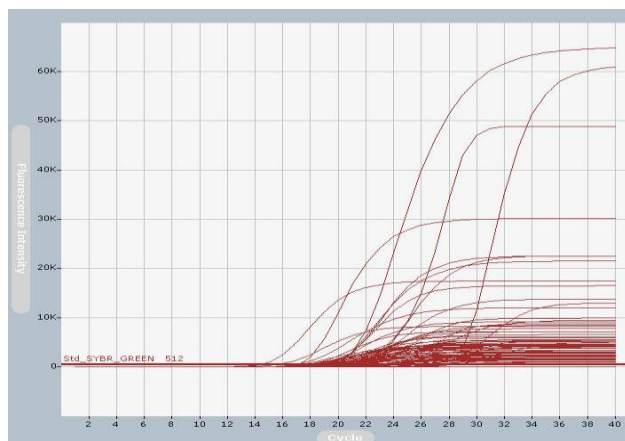


Figure (1): Amplification Plots TPMT by qPCR Samples Ct for TPMT Gene (Patient)

Gene Expression of TPMT gene Total RNA extraction was accomplished with a purity range of 1.75 to 1.95. The first stage in RT-qPCR was the synthesis of cDNA, which was followed by the amplification of the target genes. Analysis of the double Ct was used to calculate the expression of the TPMT gene, with HK gene serving as the reference gene. The amplification was recorded as having a Ct value.

Statistical analysis

Statistical analysis (SPSS), the study's data was arranged into variations and statistical descriptions (mean, SE), along with a computerized data file and frequency. According to the procedure described by (17), we employed the least significant difference (LSD) test and statistical analysis of variance one way (ANOVA) test by with a likelihood less than 0.01 ($p < 0.01$). The quantitative of level for expression of gene was measured using the method ($\Delta\Delta Ct$ relative Ct). The values of ΔCt were evaluated by measuring the Ct of the value of TPMT for all sample from the target Ct value for each sample. Then calculate the Fold inductions by using the formula $2^{\Delta\Delta Ct}$, threshold was ΔCt as cycle, ΔCt as Ct (for housekeeping gene)-Ct (for target gene), $\Delta\Delta Ct = \Delta Ct$ (for treated) - ΔCt (for con-

trol). The analysis of data was done by computer software to analyze the relative gene expression in all samples. A single sample (T-test) was used to evaluate statistically the difference between the ratios of derived expression of irradiated and the samples which non-irradiated (18).

Results and discussion

The total RNA was extracted from the Peripheral blood from each UC patients and control groups. The RNA concentration and purity were determined spectrophotometrically by measuring their absorbance at 260 (A260) and 280 (A280) by Nano spectrophotometer. Relative quantitative gene expression levels for TPMT gene in peripheral lymphocytes for UC patients regarding to their occupation using ΔCt method.

The results of distribution of samples study according to CT of the gene and the HK expression group for patient and control detected that there is gene expression was 22 patients less than the control ratio, whose value = 1, which means low gene expression, and 28 patients higher than 1, this means high gene expression, and the folding percentage was over gene expression. ($\Delta\Delta Ct = -0.26 \pm 0.43$) and (folding of gene = 8.92 ± 2.89). As shown in (Table 3).

Table (3): Distribution of Sample Study According to the TPMT Gene Expression and House Keeping

CT	HK CT	CT control average	HK control average	Δ CTE	Δ CTC	$\Delta\Delta$ Ct	Folding
24.21 ± 0.37	20.10 ± 0.27	23.48 ± 0.0	19.12 ± 0.0	4.11 ± 0.43	4.36 ± 0.0	-0.26 ± 0.43	8.92 ± 2.89

Relationship of TPMT gene expression between biological and imuran-pentaza therapy in patients

The results of this study were that the gene expression of the TPMT gene in biological therapy was more than its expression in Imuran treatment. There is no

significant difference between the gene expression of TPMT gene lower and higher than 1 p* value =NS. There is no significant difference between the gene expression of TPMT gene in patients with biological therapy and Pentaza-Imuran.

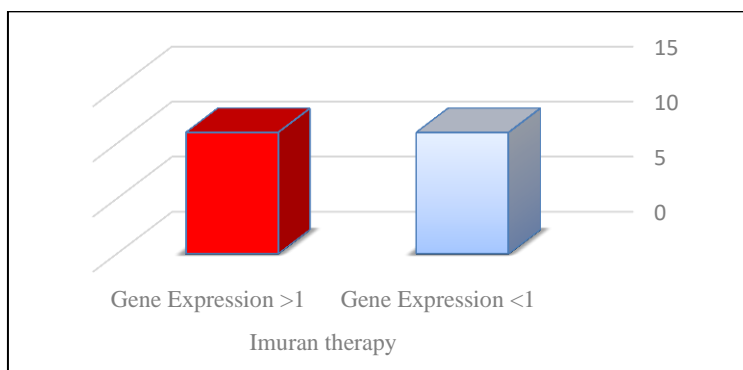


Figure (2): Gene expression of patients who take Imuran treatment

(Figure 2) shown the gene expression of patients who take Imuran treatment, and the results showed that the percentage of gene expression for the

TPMT gene was less than 1 = 11 and higher than 1 = 11, and there was no significant difference.

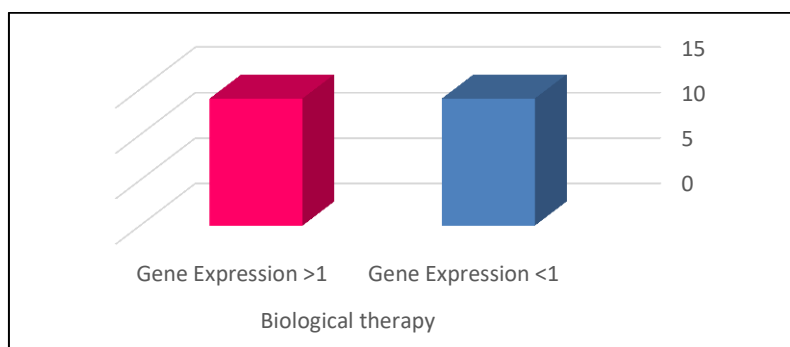


Figure (3): Gene expression of patients who take biological treatment

(Figure 3) shown the gene expression of patients who take biological therapy. The results showed that the percentage of gene expression for the TPMT

gene was less than 1 = 14 and higher than 1 = 14, and there was no significant difference.

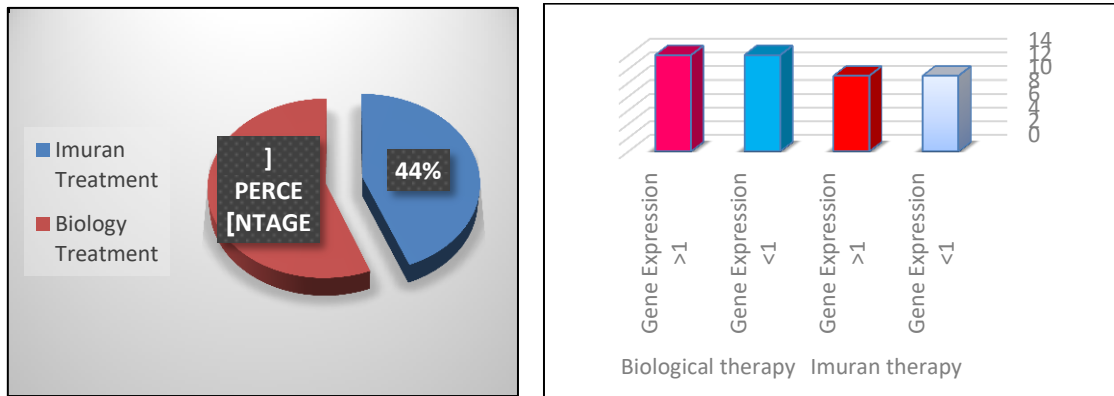


Figure (4): Gene expression of patients who take biological therapy and Imuran.

(Figure 4) shown the gene expression of patients who take biological therapy and Imuran, and the percentage of gene expression for the TPMT gene, and the percentage of biological treatment was 69% and Imuran 31%.

Furthermore, research is crucial in order to enhance the safety and efficacy of the recently discovered monoclonal antibody drugs, enabling more comprehensive precision medicine. Pharmacogenomic studies on the association among the new monoclonal antibody illicit substances and imuran are extremely significant because the mechanisms responsible for immunity and infection are very diverse. In IBD cases that have begun medical care, monitoring thiopurine metabolites and enzyme functioning enables personalized dosing based on the comprehension of pharmacogenetic biomarkers as predictors before therapy (19). The proportions of metabolites are related to the efficacy and toxicity of thiopurine. Additionally, there is a significant inter-individual variation in drug response and potential toxicity due to genetic expression in metabolizing enzymes. One of the initial pharmacogenetic examinations to be utilized in clinical practice was the TPMT. To prevent myelosuppression, start thiopurines before treatment. TPMT enzyme activity aids in dose determination, while 6-TGN and 6-MMP monitoring help de-

termine thiopurine non-response reasons.(20).

Conclusion

Immunomodulators identified as thiopurines are frequently employed in the therapeutic management of inflammatory bowel diseases, have covered the pharmacology, ways of behavior, effectiveness, optimization strategies, toxicity, and cancer risk of thiopurines this professional evaluation. In Iraqi patients with UC, the expression of the TPMT gene was significantly higher during biological therapy than over imuran medical care.

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