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Effects of Thiopurine Methyle Transferase (*TPMT*) Gene Polymorphism on Serum TPMT Levels in Iraqi Patients Diagnosed with Ulcerative Colitis

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Abstract: Ulcerative colitis is a chronic inflammatory bowel disease. Thiopurine methyltransferase (TPMT) is an enzyme that metabolizes a class of drugs called thiopurines. The aim of this study was to examine the impact of a polymorphism (rs2842934) in *TPMT* gene on TPMT level. Genotyping for *TPMT gene* (rs2842934) polymorphism was determined by the tetra-primer amplification refractory mutation system-polymerase chain reaction (TARMS-PCR). Serum TPMT level was estimated by ELIZA Technique **.**The *TPMT gene* (rs2842934) polymorphism demonstrated a significant association with UC risk. The highest prevalence of UC was seen among patients who had heterozygous GA genotype [Odd Ratio (OR): 17.51; 95% Confidence Interval (CI): (6.109-50.199); P-value <0.001]. The homozygous genotype (GG) showed the lowest risk with only (8%) in UC patients compared to the controls (72.5%) [OR: (0.03); 95%CI. (0.011-0.100); P-value < 0.001]. The highest mean TPMT enzyme level was 2071^{b}±196 pg/mL in UC patients who were homozygous for A allele compared to 709^{ab}±0.50 pg/mL in controls; P-value <0.001. Conclusions**:** The *TPMT gene* (rs2842934) polymorphism showed that the G allele was beneficial for protection from UC. In contrast, the "A" allele of rs6721961 significantly increases susceptibility to UC.

Keywords: Ulcerative Colitis, Tetra ARMS, TPMTs level and rs2842934.snp.

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Introduction

Ulcerative Colitis (UC) is a type of inflammatory bowel diseases (IBD). It is a chronic inflammation that causes ulcers, ulcerative colitis lasts for a very long time. Ulcerative colitis is a chronic inflammatory condition that affects the rectum and colon to varying degrees. The prevalence of ulcerative colitis was expected to be 5 million cases worldwide in 2023, with the frequency growing globally. symptoms are include diarrhea, bleeding, and abdominal cramping's, The colonic mucosa is inflamed(1). many factors can causes ulcerative colitis disease like the genetic factors, environmental, and immunological cases effect on the gastrointestinal tract's ability to distinguish between self and foreign antigens, that causes ulcerative colitis disease (2). UC is divided according to the severity of disease in to mild, moderate, sever. Thiopurines are administered to alleviate the symptoms of (IBD), particularly UC, because of their steroid sparing potential and efficacy in remission maintenance (3). Thiopurine methyltransferase (TPMT) is an enzyme that metabolizes medications of the thiopurines class (thiopurines include azathioprine, mercaptopurine, and thioguanine). These drugs suppress the immune system and are used to treat a range of immune-related illnesses or blood problems (for example, leukemia or organ rejection (4). The level of TPMT

activity in human tissue and therapeutic efficacy are controlled by genetic polymorphisms. The active enzyme gene is 34 kb in length, has 10 exons, and is located at 6q22.3. The TPMT pseudogene has also been identified and located on human chromosomal band 18q21.1(5) .TPMT testing entails either phenotyping (measurement of enzyme activity) or genotyping (analysis of single-nucleotide polymorphisms and mutation detection). Neither the TPMT genotype nor the phenotype alone can guarantee the identification of a TPMT deficient individual (6,7). Several studies have found that people with low or undetectable TPMT activity are at a higher risk of severe toxicity when given typical thiopurine drug dosages, even if same doses may be enough to treat people with high levels of enzyme activity. Thiopurines may not be properly digested if TPMT activity is very low, which can result in severe side effects such as decreased bone marrow function (myelosuppression) and extremely low quantities of blood cells such as red blood cells, white blood cells, and platelets (hematopoetic toxicity). This can lead to potentially fatal complications such as anemia, severe infections, and/or heavy bleeding. TPMT activity is associated with grossly abnormal thiopurine drug metabolism, excess production of cytotoxic metabolites and profound life-threatening myelotoxicity (8,9).

Materials and methods

A total 119 subjects with 61 patients diagnosed with UC and 58 healthy controls with no family history of IBD were enrolled in this study between November 2022 and March 2023. All patients were attending hospital for Gastroenterology and Hepatology Centre at the Medical City, Al-Yarmouk Teaching Hospital and Al-Imamaien Al-kadimeean Teaching Hospital. Serum was separated from blood in gel tubes by centrifugation and Human Thiopurine Methyltransferase (TPMT) level was determined by an ELISA based method. The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human TPMT in samples. DNA from peripheral blood was extracted using the blood Genomic DNA extraction kit (Geneaid Biotech) following the manufacturer's instructions. The extracted genomic DNA was stored at -20ºC until analysis. Four primers were used for tetra-primer ARMS-PCR technique with identical thermo-cycling conditions were used for genotyping. PCR protocol was as follows: initial denaturation at 95ºC for 5 min, subsequent denaturation at 95ºC for 1 min, annealing for 1 min at 60ºC for 40 cycles, extension at 72ºC for 1 min, and final extension at 72ºC for 7 min. Primer design for tetra-primer ARMS-PCR for Tetra ARMS technique was achieved using the online primer design tool http://primer1.soton.ac.uk/primer1.html. Primers are listed in Table 1. Bioneer PCR master mix was used for the amplification of the target region of the TPMT genes. The protocol was as below: 5 µL of master mix, 4 µL (primers), 3 µL template, 13 µL liter nuclease-free water with a final volume 25. Table (1) Showing Primer sequence for polymorphism detection by TETRA- ARMS.

Table (1): Primers' sequences for polymorphism detection by TETRA-ARMS

Sequence 5—3	**Product size** (bp)	**Annealing Temperature**
IF(34) TACCATTTGCGATCACCTGGAGTA	24	62.1°C
IR(34) GGATAGAGGAGCATTAGTTGCCCTC	25	63.7 °C
OF(34)ATAGCCTTACACCCAGGTCTCTGTAGTC	28	65.3 °C
OR (34)CTTGACGATTGTTGAAGTACCAGCA	25	61 °C

Statistical analysis

Analysis were performed using IBM SPSS Statistics for Windows, Version 28.0 was used to detect the effect of difference between variables. Risk ratio and 95% Confidence interval were assessed for each genotype group of patients. The study variables were compared using correlation analysis and *t*-test. The study variables were expressed as mean and standard error (mean ±SE) and standard deviation. A P-value < 0.05 was referred as statistically significant and P-value < 0.001 as statistically highly significant.

Results and discussion

TMPT enzyme level

In the present study, the average TPMT level was 1040.0±123.3 pg/mL and 706.8±19.8 pg/mL in control individuals and there was a statistically significant difference in TPMT values between UC patients and healthy individuals, P-value= 0.01 as shown in (Table 2).

Association of *TPMT* Gene Polymorphism with UC Risk

(Table 3) showing genotype distribution and allele frequencies of rs2842934 SNP in study subjects. Comparison of genotypes showed the majority of UC patients (n= 38/61-62%) were heterozygous GA unlike the controls who showed the lowest frequency for GA genotype (n=5/58-8.5%); [OR=17.51; 95% CI (6.109-50.199); *P*-value <0.001]. UC patients (n=18/61 -30%) had mutant homozygous AA genotype compared to control subjects (n=5/58-8.5%); [OR:1.78; 95% CI (0.759-4.212); *P*-value >0.05), whereas only (5/61-8%) of UC patients had wild homozygous GG genotype compared to 42(72.5%) controls [OR: 0.03; 95% CI (0.011-0.100); *P*-value <0.001). Furthermore, The G allele displayed the highest frequency (n=89-76.72%) among the healthy control subjects compared to UC patients (n=48-39.34%); P-value <0.001. A total of 74(60.66%) of UC patients were carriers of A allele compared to 27(23.28%) control subjects who had no UC; *P*-value <0.01.

Table (2): Distribution of sample study according to TPMT Enzyme level in serum of study causes

Groups	No.	TPMT mean±S.E. pg/mL	T-test	P-value
Patients	61	1040.0±123.3	2.667	0.01
Control	58	706.8±19.8		
(P≤0.01) Highly Significant				

Table (3): Genotypes and allele distributions of the TMPT rs2842934 polymorphisms among UC cases and healthy controls

Genotype rs2842934	Patients No. (%)	Control No. (%)	OR(95% ci)	P-value
GG	5(8%)	42(72.5%)	0.03(0.011-0.100)	0.0001
GA	38(62%)	5(8.5%)	17.51(6.109-50.199)	0.0001
AA	18(30%)	11(19%)	1.78(0.759-4.212)	0.18
Chi square (x^2)	6.02	45.4	-	-
Allele frequency				
G	48(39.34%)	89(76.72%)	-	-
A	74(60.66%)	27(23.28%)	-	-
P-value	0.019	0.0001	-	-
(P≤0.01) Highly Significant, (P≤0.05) Significant, NS: Non-Significant				

(Figure1) shows DNA fragments amplified with TARMS-PCR for the *TPMT* gene rs2842934 SNP were resolved from 1% agarose gel marked with a 100 bp DNA ladder at (70v/cm) for 2 hours.

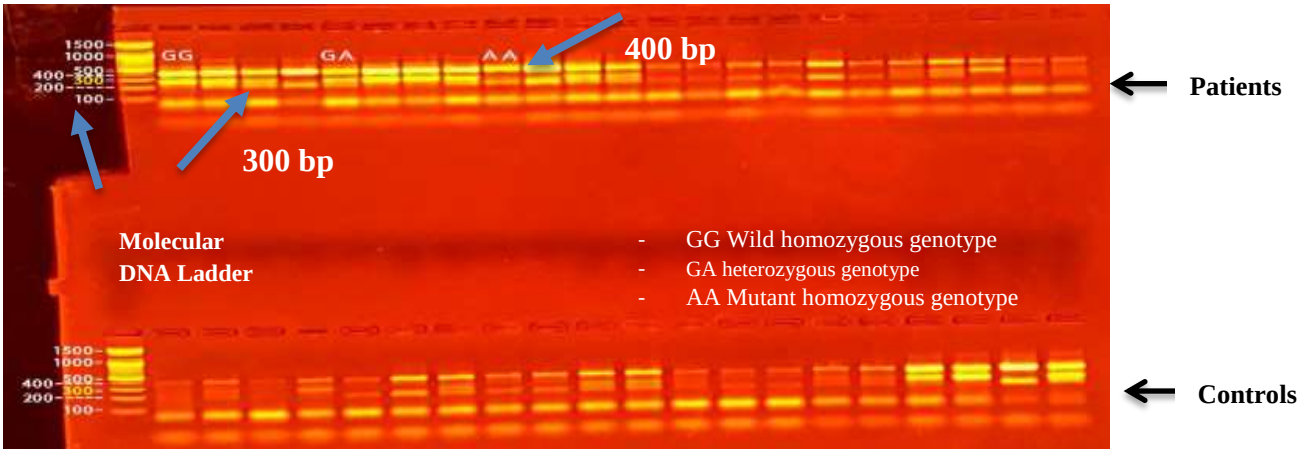

Figure (1): Gel electrophoresis of DNA fragments generated by TARMS-PCR amplification of the TPMT gene for rs2842934 SNP. PCR amplicons were loaded in 1% agarose gel for 2 hours at (70v/cm) with DNA ladder 1500 bp. DNA Fragments ranged between 100 to 400 bp in size. Out of 119 cases, (8%) of patients and (72.5%) of controls were homozygous for G allele and (62 %) of patients and (8.5%) of controls were heterozygous GA, and (30%) of patients and (19%) of controls were homozygous for A allele.

Correlation of *TPMT* gene rs2842934 SNP with serum TPMT levels

This study also investigated the impact of rs2842934 SNP in *TPMT* gene on the concentrations of TPMT in sera of the study subjects. The mean TMPT level was higher 2071^{b}±196 pg/mL in patients who had mutant homozygous AA genotype, and there was a significant difference compared to 730^{b}±38 pg/mL in control subjects with matched genotype; *P*-value <0.0001. The wild homozygous GG genotype carriers had considerably lower TPMT average1798^{b}±495 pg/mL, yet there was no significant difference compared to 730^{b}±38 in control subjects with matched genotype; *P*-value>0.01. The lowest TPMT levels 452^{a}±52 pg/mL were observed in patients who had heterozygous GA genotype, but showed no significant difference compared to 487^{a}±35 control subjects who had GA genotype; *P*-value >0.05. Results of TMPT levels in relation to genotypes of *TPMT* gene rs2842934 SNP are summarized in (Table 4).

Table (4): Distribution of TPMT enzyme level in response and non-response patients

rs2842934 Genotypes	Patients	Control	T-test	P-value
GG	1798^{b}±495	730^{b}±38	2.150	0.09
GA	452^{a}±52	487^{a}±35	-0.242	0.58
AA	2071^{b}±196	709^{ab}±0.50	6.643	0.0001
(P≤0.01) Highly Significant, NS: Non-Significant				

The pathophysiology of ulcerative colitis (UC) remains unknown but is characterized by a chronic inflammation of the gastrointestinal tract (10,11) The drug-metabolizing enzyme thiopurine methyltransferase (TPMT) catalyzes the S-methylation of thiopurines which are immunosuppressive medications that are widely used in Iraq for treating

patients with inflammatory bowel diseases, especially (Ulcerative Colitis) (12). The TPMT encoding gene is located on chromosome 6 (6p22.3) and is 34 kb long with 10 exons, eight of which encode the protein "thiopurine methyltransferase" which has a molecular mass of (28kDa) (13). Although the exonic SNPrs2842934 did not cause amino acid change in the TPMT gene previous studies demonstrated that polymorphism in the *TPMT* gene has been linked to increased enzyme levels in UC (14,15).

The current study observed significantly elevated levels of TPMT 1040.0±123.3 pg/mL in patients who had UC compared to control health subjects who developed comparable lower TPMT concentrations; *P*-value=0.01. Earlier studies, however, found no significant difference in TPMT values between individuals with and without UC (16). The *TMPT* gene rs2842934 SNP was significantly associated with an increased risk of UC in the heterozygous (GA) genotype. UC patients who had GA genotype, account for 38(62%) were 17.5 times more likely to develop UC compared to 5 (8 %) who had GG genotype; *P*-value < 0.001). Our findings also showed that the (A) allele model was associated with higher risk of UC with (60.66%) of UC patients had G allele and compared to (39.34%) who carried A allele (*P*-value = <0.05), and so G allele was considered as a risk factor for UC. Interestingly, there was no statistical significance in the homozygous mutant model (AA) where 18/61(30%) of UC patients were AA genotype carriers versus 11/58(19%) of individuals who did not have UC; *P*-value> 0.05). Accordingly the GG genotype was considered a protective genotype against UC and the G allele was deemed as a risk allele for UC in the Iraqi population. This study also found that UC patients, who had mutant homozygous AA genotype had, developed higher levels of TPMT enzyme whereas significantly lower TPMY levels were seen in UC patients who carried the risk heterozygous GA genotype. This clearly indicates that the genetic variant rs2842934 in *TMPT* gene is strongly negatively correlated with enzymatic activity of TPMT at the most frequent heterozygous GA genotype. It has been suggested that at normal levels of TPMT activity, 6-TGTP and 6-deoxyTGTP block intracellular signaling pathways and prompt lymphocytic apoptosis. A change in these activity levels inevitably results in an abnormal increase in the enzymatic activity of TPMT which in turn induces a decrease in 6-TGN and subsequently a decrease in the effectiveness of the thiopurines. Conversely, the decrease in the TPMT activity, or even a total absence of TPMT activity (as observed when TPMT exhibits polymorphisms) leads to a significant increase in the presence of 6-TGN, which will be incorporated into DNA and trigger cytotoxicity (17). Genetic variations of gene encoding TPMT enzyme involved in thiopurine metabolism give rise to varying degrees of therapeutic response and toxicity in people and subsequently predispose vulnerable people to develop UC and a more severe mucosal inflammation. contradicting findings were observed by (18) No impact of the SNP on the enzyme levels in patients with UC.

Conclusion

The *TMPT* gene rs2842934 SNP was significantly associated with an increased risk of UC and the G allele of *TMPT* gene rs2842934 SNP can be used as a factor assessing UC risk whereas the mutant homozygous AA genotype was significantly associated with higher

TPMT concentrations and thus may predict thiopurines-induced toxicity.

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