European Journal of Clinical Pharmacy
Print ISSN: 2385-409X **Online ISSN**: 3105-0409
Website: https://farmclin.com/

Original Research Article

Role of Glutathione S-Transferase (GSTM1 and GSTT1) Gene Polymorphisms in Susceptibility to Inflammatory Bowel Disease in Dhi Qar, Iraq.

Article History:

Received: 28-11-2025
Revised: 05-12-2025
Accepted: 12-01-2026
Published: 28-01-2026

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Abstract: Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic inflammatory disorders of the gastrointestinal tract. Given the crucial role of Glutathione S-Transferases (GSTs) in detoxification and mitigating oxidative stress, this study aimed to evaluate the association between the GSTM1 and GSTT1 null polymorphisms and IBD susceptibility in a localized patient cohort. Molecular analysis indicated that the combined GSTM1(-)/GSTT1(-) null genotype was significantly more prevalent in CD patients (36.67%) compared to the control group (0% or 0 individuals). The statistical analysis yielded a highly elevated Odds Ratio (OR = 35.97) with a 95% Confidence Interval (CI: 2.00 – 645.93), confirming a highly significant association with CD risk. Conversely, this strong association was not observed in UC patients. Dysfunction in these detoxification system genes, characterized by the loss of functional patterns and the predominance of the null pattern, exhibits a strong and specific correlation with Crohn's disease, underscoring a potential pathogenic mechanism for this severe form of inflammatory bowel disease.

Keywords: Inflammatory bowel disease, GSTs, GSTT1, GSTM1.

INTRODUCTION

Inflammatory bowel disease (IBD) is an autoimmune disorder characterized by chronic, non-infectious inflammation primarily affecting the lining of the gastrointestinal tract. This condition is known for its chronic, progressive, and recurrent nature, significantly impacting patients' quality of life due to impaired immunity and subsequent inflammatory dysfunction [1, 2]. IBD is classified into several predominant disorders: Crohn's disease (CD), ulcerative colitis (UC), indeterminate colitis (IC), and inflammatory bowel disease unclassified (IBD-U). While these disorders share some clinical and histopathological similarities, they affect different areas of the gastrointestinal tract: UC primarily targets the colonic mucosa in a continuous pattern, whereas CD can affect any part of the gastrointestinal tract, from mouth to anus, in a discontinuous (intermittent) pattern [1, 3]. The incidence and prevalence of IBD have increased significantly over the past decades since its emergence in the 20th century, particularly in industrialized countries [4]. This variation across different populations suggests that genetic and environmental factors are vital areas for molecular research. Indeed, although the exact etiology of IBD remains complex and multifactorial, it is well-established that a combination of genetic and environmental factors leads to the uncontrolled activation of the intestinal immune system against commensal bacteria and other targets [5, 6].

Regarding genetic factors, they appear fundamental to IBD development, with nearly 200 genetic loci having been linked to disease predisposition through genom wide association studies (GWASs) [6]. Concerning environmental factors, the marked increase in prevalence in industrialized countries highlights the significant role of environmental influences. Among

these, smoking, alcohol, drug use, and diet are considered the most important. The hypothesis suggests that these factors affect the lining of the digestive tract and disrupt the gut microbiome a complex ecosystem of microorganisms inhabiting the GI tract. Imbalances in the gut microbiome are considered prominent environmental triggers, potentially contributing to the development of chronic inflammation [7, 8]. Glutathione S-Transferases (GSTs) (where the 'S' refers to the sulfur atom) are a family of multifunctional enzymes with detoxification properties. GSTs catalyze the conjugation of mutagenic electrophiles and oxidized lipids with cellular glutathione, yielding hydrophilic and less toxic metabolites [9]. GSTs, formerly known as ligandins, belong to a class of Phase II metabolic isoenzymes in prokaryotes and eukaryotes [10]. The most clinically significant members of the GST family are GSTT1 and GSTM1. An important genetic polymorphism has been described in the GSTM1 gene, located on chromosome 1p13.3, which is a partial deletion resulting in the GSTM1null genotype and the complete absence of enzyme activity. A similar deletion polymorphism has been reported in the GSTT1 gene, located on chromosome 22q11.2 (the GSTT1null genotype) [11, 12]. Because of the critical role of GSTs in oxidative stress mitigation and detoxification of carcinogens, GSTM1 and GSTT1 allelic polymorphisms may lead to less efficient clearance of toxic intestinal metabolites, thus modifying susceptibility to UC and CD [13, 14].

The aims of the study

This study aimed to investigate the association of GSTM1 and GSTT1 deletion polymorphisms with Inflammatory Bowel Disease (IBD) susceptibility in patients from Dhi Qar Governorate, Iraq. This was achieved through the molecular detection of the null genotypes of GSTT1 and GSTM1, providing region-specific data to elucidate their impact on IBD susceptibility and pathogenesis.

METHODOLOGY

The sampling period extended from November 2023 to May 2024. (90) blood samples were collected from private clinics, Nasiriyah General Hospital, and Shatra Hospital in Dhi Qar Governorate for diagnosed patients, with (30) samples for Crohn's disease patients and (30) samples for ulcerative colitis patients, which represent the current study group, and (30) blood samples for healthy people from different segments of society. The exclusion of cases that failed to meet the study criteria or were not disease-associated was conducted subsequent to securing all necessary official approvals, acquiring full informed consent from patients, and in compliance with patient privacy protocols. The exclusion of cases that failed to meet the study criteria or were not disease-associated. Our study protocol is consistent with the ethical principles of the 1975 Declaration of Helsinki, The study's implementation in the health department institutions was approved by the research ethics committees of the Thi-Qar Health Department **(**Document 119/2025, dated 3/23/2025**)**, and informed consent was obtained from each patient included in the study.

Samples Collection :

A total of 90 blood samples were collected from diagnosed patients and healthy controls in Dhi Qar Governorate, sourced from private clinics, Nasiriyah General Hospital, and Shatra Hospital. The cohort comprised three distinct groups: 30 patients with Crohn's disease, 30 patients with ulcerative colitis, and 30 healthy individuals from the community. 5 ml of venous blood was taken from both the patient and healthy groups. The blood samples were placed in tubes containing the anticoagulant EDTA and stored at -20°C.

DNA Extraction

DNA extraction and purification were performed using the gSYNC™ Genomic DNA Kit (Genead, Taiwan) strictly according to the manufacturer's instructions. Following assessment of concentration and purity, the extracted DNA was stored at -20°C until genotype determination. The integrity and presence of the DNA were subsequently verified by agarose gel electrophoresis, following the methodology described by Sambrook et al. (1989).

Multiplex Polymerase Chain Reaction technique (MPCR)

MPCR technique was performed to amplify both GSTT1 and GSTM1 genes based on the method (Dermawan, 2021), using the albumin gene as an internal control as well as in the detection of products. This technique includes the following materials (extracted DNA, Master mix, Double – distilled water (ddH2O) three pairs of primers.

PCR Reaction

The Multiplex Polymerase Chain Reaction (MPCR) technique was optimized to allow the simultaneous amplification of multiple specific DNA targets in a single reaction tube. The method was performed using a final reaction volume of 50 µl. Each reaction tube contained the following components: 25 µl of Master Mix (a pre-mixed solution containing the basic reagents for DNA amplification), 5 µl of DNA template and the primers for the three targeted genes (GSTT1, GSTM1, and Albumin). Specifically, 1 µl of forward primer and 1 µl of reverse primer were added for each gene pair. The final volume was achieved by adding 18 µl of nuclease-free water. Upon preparation, the samples were transferred to a thermal cycler and run according to the optimized cycling program .

Statistical Analysis

Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 25. The analysis of variance (ANOVA) test was employed, which indicated that the observed differences in group means were statistically significant ($p \leq 0.05$).

RESULTS

Polymorphisms (Positive, Null) of the genes GSTM1 and GSTT1 :

Comparison of the genotypes of the GSTM1 and GSTT1 genes in the two groups (Crohn's disease patients and the control group). The results of this study on the genotypes of the GSTM1 and GSTT1 genes, both positive and negative (+, -) and indicated in (Table 1), revealed the following: For the GSTM1 gene (+), the positive pattern was found in (40%) 12 Crohn's patients compared to (86.67%) 26 of the control group, which means that the presence of the gene is more common in healthy people and may be a protective factor against the disease. In contrast, the GSTM1(-) negative pattern appeared in 18 (60%) Crohn's disease patients compared to 4 (13.33%) of the comparison group, with an odds ratio (OR = 9.75) and (95% CI: 2.70–35.11), indicating that the absence of the gene increases the risk of Crohn's disease by about ten times, and in a statistically significant manner. As for the GSTT1 gene, the positive (+) pattern was found in 21 (70%) of Crohn's patients and in 30 (100%) of the comparison group, indicating that the presence of this gene is very common in healthy people and may be associated with a lower risk of infection. The negative GSTT1(-) pattern appeared in (30%)9 of Crohn's disease patients and was not present in the comparison group (0%)0, with an odds ratio (OR = 26) and (Cl: 1.48–68.55), indicating a strong association between the negative pattern and increased risk and a clear statistical significance.

Table (1) Genotypes of the GSTM1 and GSTT1 genes for the Crohn's disease patients and control groups

Genotype	Control Group N=30 (%)	Crohn's Disease Group N=30 (%)	OR	95% CI
GSTM1(+)	26 (86.67%)	12 (40%)	1.0	—
GSTM1(-)	4 (13.33%)	18 (60%)	9.75	2.70 – 35.11
Total	30 (100%)	30 (100%)		
GSTT1(+)	30 (100%)	21 (70%)	1.0	—
GSTT1(-)	0 (0%)	9 (30%)	26	1.48 – 68.55
Total	30 (100%)	30 (100%)		
OR: Odds Ratio 95% CI: 95% Confidence Interval				

Comparison of the shared genotypes of the GSTM1 and GSTT1 genes in the Crohn's disease and control groups

The combined genotypes of the GSTM1 and GSTT1 genes, as summarized in Table (2), revealed a distinct difference in distribution. The double positive pattern (GSTM1(+) / GSTT1(+)) was found in (19) Crohn's disease patients (63.33%) compared to (30) individuals (100%) in the control group (OR = 1.0). This finding suggests that the presence of both functional genes is significantly more prevalent among healthy individuals and may therefore serve as a protective factor. Conversely, the double negative pattern (GSTM1(-) / GSTT1(-)) appeared in (11) Crohn's patients (36.67%) but was not recorded in the control group (0 individuals or 0%). Statistical analysis yielded a highly elevated Odds Ratio (OR = 35.97) with a 95% Confidence Interval (CI: 2.00 – 645.93), indicating that the absence of both functional genes greatly increases susceptibility to Crohn's disease.

Table (2) The common genotypes of the GSTM1 and GSTT1 genes for the two groups of Crohn's disease patients and the control group.

Genotypes	Control Group N=30 (%)	Crohn's Disease Group N=30 (%)	OR	95% CI
GSTM1(+)\GSTT1(+)	30 (100%)	19 (63.33%)	1.0 (Ref)	—
GSTM1(-)\GSTT1(-)	0 (0%)	11 (36.67%)	35.97	2.00 – 645.93
Total	30 (100%)	30 (100%)		
OR: Odds Ratio	**95% CI: 95% Confidence Interva**			

Comparison of GSTM1 and GSTT1 genotypes in ulcerative colitis patients and controls.

The results in Table (3) for the genotypes of the GSTM1 and GSTT1 genes for both positive and negative patterns have shown that for the GSTM1 gene, the positive pattern (+) was found in (60%) 18 of the ulcerative colitis patients compared to (86.67%) 26 of the comparison group, which means that the presence of the gene is more common in healthy people and may have a protective role. While GSTM1 (-) negative pattern appeared in (40%)12 of ulcerative colitis patients versus (13.33%)4 of the comparison group, with an odds ratio (OR = 4.33) and (Cl: 1.20–15.60), which is statistically significant and indicates that the negative pattern of the gene increases the risk of the disease by

about four times. As for the GSTT1 (+) gene, the positive pattern appeared in (86.67%)26 of the ulcerative colitis patients and in (100%)30 of the comparison group, while the GSTT1 (-) negative pattern was found in (13.33%)4 of the ulcerative colitis patients and no appearance of the gene was recorded in the comparison (0%)0, with (OR = 10.35) and (Cl: 0.53–98), and this result is not statistically significant because the confidence interval includes the value 1.

Table (3) Genotypes of the GSTM1 and GSTT1 genes for the ulcerative colitis patients and the control group

Genotype	Control Group N=30 (%)	Ulcerative Colitis Patient Group N=30 (%)	OR	95% CI
GSTM1(+)	26 (86.67%)	18 (60%)	1.0	—
GSTM1(-)	4 (13.33%)	12 (40%)	4.33	1.20 – 15.60
Total	30 (100%)	30 (100%)		
GSTT1(+)	30 (100%)	26 (86.67%)		—
GSTT1(-)	0 (0%)	4 (13.33%)	10.35	0.53 – 98
Total	30 (100%)	30 (100%)		
OR: Odd Rations		95% CI Confidence Interval		

Comparison of the common genotypes of GSTM1 and GSTT1 genes in the ulcerative colitis patients and control groups.

were analyzed. The double positive pattern (GSTM1(+) / GSTT1(+)) was found in (26) ulcerative colitis patients (86.67%) compared to (30) individuals in the control group (100%). With an Odds Ratio (OR = 1.0), this suggests that the presence of both functional genes is more common among healthy individuals and may therefore represent a protective factor. Conversely, the double negative pattern (GSTM1(-) / GSTT1(-)) appeared in (4) ulcerative colitis patients (13.33%) but was not recorded in the comparison group (0% or 0 individuals). The statistical analysis yielded an Odds Ratio (OR = 10.36) with a 95% Confidence Interval (CI: 0.53 – 201.45). Crucially, this association was not statistically significant. The wide confidence interval, likely due to the small sample size and the zero cell, indicates that the absence of both genes does not significantly increase the risk of infection in the UC cohort examined.

Table (4) Common genotypes of the GSTM1 and GSTT1 genes for the two groups of ulcerative colitis patients and the control group

Genotype	Control Group N=30 (%)	Ulcerative Colitis Patient Group N=30 (%)	OR	95% CI
GSTM1(+)\GSTT1(+)	30 (100%)	26 (86.67%)	1.0	—
GSTM1(-)\GSTT1(-)	0 (0%)	4 (13.33%)	10.36	0.53 – 201.45
Total	30 (100%)	30 (100%)		
OR: Odd Rations	**95% CI Confidence Interval**			

Polymorphisms of the GSTT1 and GSTM1 genes

The genotypes of both GSTM1 and GSTT1 were examined at the genome level using polymerase chain reaction (PCR). PCR amplification products of 216 bp for GSTM1 and 480 bp for GSTT1 were detected in normal samples, while they were not present in individuals carrying the null allele. (Figure 1) DNA bands of PCR amplification products of the GSTM1, GSTT1, and control beta-globin genes are shown, separated by electrophoresis on a 2% agarose gel. The frequencies of GSTM1 and GSTT1 deletion polymorphisms were compared between IBD patients and controls. Comparative analysis revealed a significantly higher prevalence of homozygous GSTM1 genotype in Crohn's disease patients (60%) compared to the control group (13.33%), odds ratio (OR = 9.75), 95% confidence interval (CI = 2.70-35.11), While ulcerative colitis patients showed a lower but still high prevalence (40% vs. 13.33%, odds ratio = 4.33, 95% confidence interval = CL (1.20-15.60). For GSTT1, all controls were positive, compared to 70% of Crohn's disease patients (OR = 26, 95% CI = 1.48–68.55) and 86.67% of ulcerative colitis patients (OR = 10.35, 95% CI = 0.53–98). The combination GSTM1 (+) / GSTT1 (+) was prevalent in controls (100%), However, it was decreased in Crohn's disease (63.33%) and ulcerative colitis (UC) patients (86.67%) while GSTM1/(−) GSTT1(−) was absent in controls, but was observed in (36.67%) of Crohn's disease patients and (13.33%) of ulcerative colitis patients. The GSTM1(−) genotype showed a stronger association with Crohn's disease than with ulcerative colitis, suggesting a possible difference in the genetic risk profile between patients.

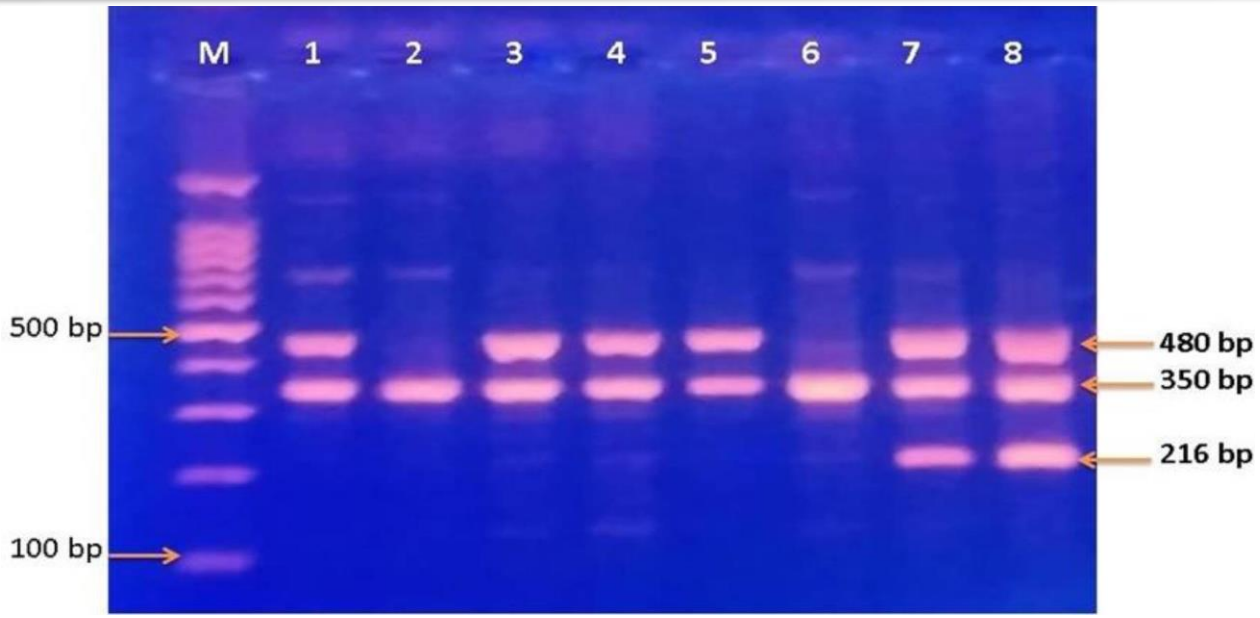

Figure (1) Electrophoresis of the GSTM1 and GSTT1 genes on agarose gel, concentration 2%:M standard DNA (100 pb), sequences 5-4-3-1 lack the GSTM1 gene, sequences 6-2 lack both genes, sequences 8-7 contain both genes.

DISCUSSION

Inflammatory bowel diseases (IBDs), including ulcerative colitis and Crohn's disease, are chronic inflammatory disorders of the gastrointestinal tract, most often diagnosed in adolescence and young adulthood, with an increasing incidence in children [15], IBD is usually diagnosed between the ages of 20 and 40. However, very young patients with IBD may have different disease mechanisms compared to elderly patients, which may affect prognosis and healthcare (16). Glutathione transferases (GSTs) are multifunctional enzymes involved in the management of cellular oxidative stress, catalyzing the conjugation of glutathione to endogenous and exogenous compounds. They are of broad importance in relation to human susceptibility or resistance to multifactorial human diseases [17]. GSTM1, GSTT1, and GSTP1 are genes that encode common GST proteins [18]. Glutathione enzymes detoxify exogenous and endogenous electrophilic molecules that interact with essential biological molecules such as DNA[19]. Our results revealed a significantly higher homozygous genotype rate for the GSTM1 gene in Crohn's disease patients (60%) compared to the control group (13.33%), while ulcerative colitis patients showed a lower prevalence (40% vs. 13.33%). For GSTT1, all controls were positive, compared to 70% of Crohn's disease patients and 86.67% of ulcerative colitis patients, The frequencies of GSTM1 and GSTT1 gene deletions may vary according to the ethnicity and continental origin of the studied population, with implications for achieving the goal of precision/personalized medicine in clinical practice. The prevalence of heterozygous GSTM1 and GSTT1 genotypes was higher in patients than in healthy volunteers. Conversely, a higher prevalence of heterozygous genotypes was observed in healthy volunteers from East Asia [20]. Our results showed that the combination GSTM1(+)/GSTT1(+) was prevalent in controls (100%), but decreased in Crohn's disease (63.33%) and ulcerative colitis (UC) patients (86.67%), while GSTM1(−)/GSTT1(−) was absent in controls, but was observed in (36.67%) of Crohn's disease patients and (13.33%) of ulcerative colitis patients, The absence of the functional gene GSTT1 does not play an important role in the pathophysiology and development of inflammatory bowel diseases, ulcerative colitis, and Crohn's disease in the Iranian population, While the GSTM1 null genotype could be considered a potential genetic factor that increases susceptibility to inflammatory bowel diseases and ulcerative colitis, a significantly higher frequency of the GSTM1 null genotype was found in IBD patients compared to controls, suggesting its potential role in IBD susceptibility [20]. Current evidence suggests that the risk of developing environmentally induced inflammatory bowel disease (IBD) can vary among individuals carrying certain genetic variants [18]. The GSTM1(−) genotype showed a stronger association with Crohn's disease than with ulcerative colitis, suggesting a possible difference in genetic risk profile between patients, the GSTM1null genotype was significantly more common in ulcerative colitis patients than in the control group ,suggesting a potential role in ulcerative colitis susceptibility, However, no significant associations were found between the GSTT1 gene in ulcerative colitis or either gene in Crohn's disease, possibly due to limited sample size. These findings suggest that GSTM1 deletion may contribute more to ulcerative colitis than Crohn's disease, highlighting subtype-specific genetic influences in inflammatory

bowel disease [21]. The null genotype of genes such as GSTM1 and GSTT1 indicates the complete absence of the corresponding functional enzyme. This absence translates into a decreased overall capacity for detoxification and resistance to oxidative stress. In IBD patients with this genotype, they may be more susceptible to cellular damage caused by chronic inflammation, which may contribute to the onset or progression of the disease [22].

CONCLUSION

This study successfully evaluated the relationship between the null polymorphisms in the Glutathione S-Transferase M1(GSTM1) and T1(GSTT1) genes and susceptibility to Inflammatory Bowel Diseases (IBD), specifically Crohn's disease (CD) and ulcerative colitis (UC). Our descriptive results revealed clear differences in genotype distribution across the groups. Notably, the GSTM1 null genotype was significantly more prevalent in Crohn's disease patients (60.00%) compared to ulcerative colitis patients (40.00%) and the control group (13.33%). Similarly, the GSTT1 null genotype was present in (30.00%) of CD patients, contrasting with its complete absence in the control group (0.00%). Crucially, statistical analysis revealed a highly significant association between the GSTM1 and GSTT1 null genotypes and an increased risk of Crohn's disease (CD). However, the association with ulcerative colitis (UC) was not found to be statistically significant. This finding strongly suggests that impaired activity of these essential detoxification enzymes, which catalyze the conjugation of glutathione (GSH) with xenobiotics and toxic endogenous metabolic products, plays a specific pathogenic role in the development of CD.

Acknowledgments

We would like to express our deepest gratitude to the medical staff at Dhi Qar Hospital for their efforts in facilitating our fieldwork. We also extend our sincere thanks to all the sample donors, whose valuable contributions were a cornerstone of this research.

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