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Original article

Impact of GST (M1, T1) gene polymorphisms on hematobiochemical changes in *Helicobacter pylori*-infected patients

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ABSTRACT

Background: Infection by *Helicobacter pylori* (*H. pylori*) is prevalent and linked to many pathological conditions. Numerous studies have explored the association between *H. pylori* and gastric diseases. However, there are fewer studies on the impact of *H. pylori* on biochemical and hematological parameters. Furthermore, the role of genetic variations in mediating these hematobiochemical alterations remains poorly unrevealed. **Objective:** explore the association between Glutathione S-transferase mu1 and theta1 (GST M1, T1) gene polymorphisms and hematobiochemical changes in *H. pylori*-infected individuals. **Subjects and methods:** Thirty *H. pylori*-infected male patients and thirty healthy uninfected males as control were enrolled in the study. Biochemical profiles, oxidative stress markers, and hematological profile were measured, along with glutathione S-transferase mu1 and theta1genotyping. The statistics were conducted using the SPSS program (version 27). **Results:** *H. pylori*-infected patients had significantly decreased mean levels of many parameters like hemoglobin. Meanwhile significantly increased levels of other parameters like monocytes in comparison to the non-infected control. Additionally, patients with the null genotype for mu1 and theta1 (double null) had significantly higher levels of monocytes and other parameters. However, they had reduced total glutathione S-transferase enzyme activity compared to the control group. **Conclusion:** This study offers proof of hematobiochemical alterations related to infection with *H. pylori* and underscore the modulatory role of GSTM1/GSTT1 genetic variation with a higher susceptibility in null genotypes carriers.

Introduction

Helicobacter pylori (*H. pylori*), a Gram-negative, microaerophilic, and spiral-shaped bacterium, is a common human pathogen found worldwide, infecting over half the global population [1-3]. Transmission likely occurs through oral-fecal or oral-oral routes, with the bacteria found in saliva and stool [4]. Diagnosis involves both invasive (endoscopy-based) and non-invasive (stool antigen, serum antibody) techniques [5]. *H. pylori* colonizes the stomach lining early in life, persisting through chronic inflammation by disrupting intracellular epithelial processes [2,6]. Immune cells release reactive oxygen species (ROS) and cytokines in response to inflammation, leading to oxidative

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stress. Additionally, *Helicobacter pylori* infection generates reactive nitrogen species (RNS) and other byproducts that induce oxidative stress, damaging cellular components and promoting inflammation [7,8]. Glutathione S-transferases (GSTs) are ubiquitous enzymes in gastrointestinal epithelial tissues. These enzymes play crucial roles in cellular defense, including detoxification of harmful substances. GSTs catalyze the conjugation of glutathione with electrophilic compounds, rendering them water-soluble for elimination [9,10]. Within the GST superfamily, two key genes named GSTM1 and GSTT1 have attracted significant attention due to their potential role in various diseases. Individuals with a "positive" genotype have both active copies of the gene, while those with a "null" genotype lack both copies, leaving them with less enzymatic activity [11]. This can affect how efficiently they detoxify harmful substances, potentially increasing their risk of certain illnesses. Recent research suggests that genetic variations in these genes can lead to decreased enzyme activity, potentially affecting our body's ability to fight off harmful substances and contributing to disease development [12]. Genetic variations in detoxification enzymes like GSTs, particularly null genotypes for M1 and T1, might increase the risk of severe *H. pylori* infection outcomes like gastric cancer. These polymorphisms potentially reduce the body's ability to neutralize harmful substances produced by *H. pylori*, leading to increased damage to the stomach lining. However, research on the exact impact of GST polymorphisms on gastritis, ulcer development, and overall disease severity is still ongoing [13,14]. This study aimed to explore the association between GST (M1, T1) gene polymorphisms, enzyme activity, oxidative stress status, and hematobiochemical changes in patients infected by *H. pylori*.

Subjects and methods

1. Study design

This case-control observational study enrolled a total of 60 male participants.

2. Study population

Thirty randomly selected male patients infected by *H. pylori* and 30 uninfected healthy males as control were recorded in this study. Both groups were matched for sex, age, smoking habit (all participants are non-smokers), and body mass index (BMI). Based on GSTM1/GSTT1 genotypes, control and infected groups were further divided into positive and null sub-groups (A; control/positive, B; control/null, C; infected/positive and D; infected/null). Individuals with diabetes, hypertension, renal or hepatic diseases, parasitic worm infestation, or any chronic diseases affecting hematobiochemical parameters were excluded. All participants gave their consent to be involved in the work, this study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Institutional Review Board of the Faculty of Medicine at Zagazig University on August 20, 2023 (protocol code ZU-IRB#:101034-20-8-2023).

3. Sample collection and preparation

Each participant gave 8 milliliters of venous blood drawn. The blood was divided into three different tubes. The first two tubes were sterile vacutainers containing ethylenediaminetetraacetic acid (EDTA-K3). In each case, one tube was used for the determination of hematological parameters, and the other tube was used for DNA extraction. The third tube was a sterile plain vacutainer, intended for biochemical analysis and enzymatic assays after clotting and centrifugation. In addition to blood samples, participants also provided random stool samples.

4. Laboratory measurements

4.1 Hematological parameter determination

A complete blood count (CBC) was obtained using an automated hematology analyzer (model XN 2000, Sysmex, Japan) [15]. Additionally, differential leukocyte counts were determined by examining Leishman-stained peripheral blood smears [16]. Hematological parameters, including hemoglobin, hematocrit, RBCs (red blood cells), total and differential white blood cell counts (WBCs), and platelets, were measured. MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), and MCHC (mean corpuscular hemoglobin concentration) were calculated from the hematological index data.

4.2 Biochemical parameter determination

Liver function tests including alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin, alkaline phosphatase and gamma-glutamyl transferase (GGT) in addition to lactate dehydrogenase, creatinine, magnesium, calcium, and iron were analyzed using the fully automated analyzer Cobas c311/501 (Roche Diagnostics, Germany) [17]. Serum electrolytes,

including sodium, potassium, ionized calcium, chloride, and blood pH, were analyzed using an electrolyte autoanalyzer (Cornley-K-Lite 5, Meizhou, Cornley Hi-Tech Co., Ltd., China) [18].

4.3 Oxidative markers determination

Glutathione S transferase (GST) activity and malondialdehyde (MDA) levels were determined colorimetrically, in accordance with previously reported methods [19, 20], by a plate reader (BMG Labtech, FLU Ostar Omega, Germany) using a commercial kit (Biodiagnostic, Egypt). The results were expressed in IU/L.

4.4 *Helicobacter pylori* stool antigen

Participants were provided with hygienic, leak-proof containers to deliver fresh stool samples, within two hours of stool collection, a lateral flow immunochromatographic analysis was performed to determine the presence of *H. pylori* antigen in the supplied stool samples. The results were classified as either *H. pylori* antigen-positive or negative based on the presence of the color produced. (Right Sign, Hangzhou Biotest Biotech Co., Ltd., Hangzhou, China) [21].

5. DNA extraction

Using INtRON Biotechnology-Korea's G-Spin Column complete DNA extraction kit, genomic DNA was extracted from 60 participants using a 200-microliter whole blood samples according to manufacturer protocol, the purity of the isolated DNA was checked on 1% agarose gel electrophoresis [22].

6. GSTM1 / GSTT1 Genotyping

GSTM1/GSTT1 genotyping was determined by multiplex PCR using Techne TC-4000 thermal cycler (Techne Ltd., UK). In a 12.5 µL total reaction volume. 6.25 µL of 2X master mix (Trans Gen Biotech Co., Ltd. China), 2.85 µL of nuclease-free water, 75 ng of DNA template, and 0.15 µL primers of GSTM1 forward (5’ GAA CTC CCT GAA AAG CTA AAG C 3’), GSTM1 reverse (5’ GTT GGG CTC AAA TAT ACG GTG G3’), GSTT1 forward (5’ TTC CTT ACT GGT CCT CAC ATC TC 3’), and GSTT1 reverse (5’ TCA CCG GAT CAT GGC CAG CA 3’). as internal control exon 7 of CYP1A1 was coamplified using (5’ GAA CTG CCA CTT CAG CTG TCT 3’) as forward primer and (5’ CAG CTG CATTTG GAA GTG CTC 3’) as reverse primer [23]. A denaturation step was performed at 94°C for five minutes at the start of the process. The next step involved 32 cycles of denaturation (94°C for 1 minute), annealing (59°C for 1 minute), extension (72°C for 1 minute), with a final extension step at 72°C for 10 minutes. The amplified DNA fragments are then examined on a 2% ethidium bromide-stained agarose gel. Positive genotypes (wild types) were identified by the presence of bands at both 215 bp (GSTM1) and 480 bp (GSTT1). Conversely, null genotypes were identified by the absence of these bands [23].

7. Statistical analysis

Statistical software (SPSS, version 27) was used for data analysis. Qualitative data were expressed as frequencies and percentages, whereas continuous data were shown as mean values with standard deviations (SD). Data normality was assessed using the Shapiro-Wilk test, this test determines if a data set comes from a normally distributed population. Comparison of hematobiochemical parameters between *H. pylori*-infected patients and control individuals was done by independent samples t-test as the data were normally distributed. Comparisons between the four subgroups were conducted using one-way ANOVA for parametric data and the Kruskal-Wallis test for non-parametric data. Levene's test confirmed homogeneity of variance for parametric analyses. To determine specific group differences, post-hoc Tukey HSD tests were applied for ANOVA, while Dunn's test was used for pairwise comparisons following the Kruskal-Wallis test. Differences were considered statistically significant at $p < 0.05$.

Results

Demographic data

In this observational case-control study, 30 individuals infected with *Helicobacter pylori* were randomly selected, and 30 healthy controls were enrolled. All participants were volunteer males, residing in the same area, and shared similar nutritional and lifestyle backgrounds. **Table 1** presents an overview of the demographic details of the study participants, reflecting similarities between the study groups.

Hematological parameters results

Table 2 presents the evaluated hematological parameters in the study groups. A significant decrement in hemoglobin (14.1±0.8 vs 14.5±0.8 g/dL), hematocrit (40.9±2.5 vs 42.4±2.9 %), and mean corpuscular volume (80.9±4.8 vs 83.9±4.1 fL) was reported in the infected group in comparison to the control group, with p-values of 0.024, 0.042, and 0.011, respectively.

The infected group had higher monocyte counts (0.34±0.15 x103/µL) than the control group (0.32±0.11 x103/µL) (p=0.003). Infected patients also had greater basophil (0.05±0.02 vs 0.04±0.01 x103/µL) and eosinophil (0.23±0.08 vs 0.17±0.06 x103/µL) counts (p=0.004 and p=0.002, respectively).

Biochemical parameters results

The evaluated biochemical parameters in the study groups are presented in **Table 3**. Serum calcium level was significantly lower in the infected group (9.16±0.53 mg/dL) compared to control individuals (9.45±0.23 mg/dL) (p = 0.009). Iron levels were also reduced in infected individuals (66.1±25.1 µg/dL) relative to control participants (85.9±24.48 µg/dL) (p = 0.003). Additionally, the lipid peroxidation indicator malondialdehyde (MDA) was increased in the infected group (11.37±4.09 IU/L) versus control one (8.96±2.00 IU/L) (p = 0.006).

GSTM1, GSTT1 distribution among the studied groups

GSTM1, GSTT1 genotypes and combined genotypic distribution among the studied groups are presented in **Table 4**. Our data revealed an equal distribution of GSTM1 genotype across both control and infected groups. In particular, 46.7% of individuals were GSTM1-positive, while 53.3% were GSTM1-null. Similarly, GSTT1 genotypes distribution did not differ significantly between studied groups, with 50% of individuals being GSTT1-positive and the remaining 50% harboring GSTT1-null genotype. Furthermore, non-significant variations were observed in the combined GSTM1/GSTT1 genotype distributions between the studied groups. Both infected and control group exhibited an equal prevalence (30%) of the double-null genotype.

GSTM1, GSTT1 distribution among the studied population

Results of GSTM1, GSTT1 genotypes and combined genotypic distribution in the whole study population are presented in **Table 5**. Comparable distributions of GSTM1-positive (46.7%) and GSTM1-null (53.3 %) genotypes were observed. GSTT1 positive and GSTT1 null genotypes were equally distributed with 50 % for each genotype. Results showed non-significant differences in combined genotypic distributions, with higher incidence of double null genotype (30%).

Association between altered parameters and GSTM1 gene polymorphism

Table 6 presents the association between blood parameters and GSTM1 genotype in the study groups. Hemoglobin, hematocrit, MCV, total calcium, and iron levels were comparable across all genotypes. However, *H. pylori*-infected individuals with the GSTM1 null genotype exhibited significantly higher monocyte, eosinophil, and basophil counts compared to the control groups (p <0.05). Additionally, MDA levels were elevated in *H. pylori*-infected GSTM1 null individuals in comparison to the control individuals. Notably, GST activity was significantly lower in GSTM1 null control individuals compared to GSTM1-positive controls (p <0.05).

Association between altered parameters and GSTT1 gene polymorphism

Table 7 displays the association between altered parameters and GSTT1 gene polymorphisms in study groups. The results showed that monocytes, eosinophils, and basophils counts were considerably higher in the infected group with null GSTM1 genotype compared to the controls group with positive and null GSTT1 genotypes(p<0.05). Furthermore, MDA levels were elevated in infected individuals with the GSTT1 null genotype compared to the control with a positive or null GSTT1 genotypes (p<0.05). Lastly, the mean GST levels were significantly lower in the infected individuals with a null GSTT1 genotype in comparison to the controls harboring null or positive GSTT1 genotypes (p<0.05).

Table 1. Demographic data of the study population

Parameter	Control (n=30)	Infected (n=30)	P- value
Age (years)	41.4±8.9	41.9±7.8	NS
BMI (Kg/m2)	27.7±3.2	26.7±2.9	NS
Data presented as mean ± standard deviation, BMI (body mass index), n (number), NS (non-significant)			

Table 2. Evaluated hematological parameters in the study groups

Hematological Parameters	Reference range	Control (N=30)	Infected (N=30)	P-value
Hemoglobin (g/dL)	13.2-17.3	14.5±0.8	14.1±0.8	0.024*
RBC (x106 /µL)	4.3-5.7	5.1±0.40	5.1±0.43	NS
Hematocrit (%)	39-49	42.4±2.9	40.9±2.5	0.042*
MCV (fL)	80-100	83.9±4.1	80.9±4.8	0.011*
MCH (pg)	27-34	28.9±2.0	28.0±2.2	NS
MCHC (g/dL)	32-37	34.4±1.7	34.4±1.2	NS
Platelets (x103/µL)	150 - 450	247±39	229±42	NS
WBC (x103/µL)	4 – 10	6.5±1.7	7.0±2.1	NS
Neutrophils (x103/µL)	2 – 7	3.4±1.1	3.5±1.5	NS
Lymphocytes (x103/µL)	1 – 3.5	2.6±0.8	2.7±1.1	NS
Monocytes (x103/µL)	0 – 1.0	0.32±0.11	0.34±0.15	0.003*
Eosinophils (x103/µL)	0 – 0.5	0.17±0.06	0.23±0.08	0.002*
Basophils (x103/µL)	0 – 0.1	0.04±0.01	0.05±0.02	0.004*

Data presented as mean ± standard deviation), RBC (red blood cell), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin), MCHC (mean corpuscular hemoglobin concentration), WBCs (white blood cells). *Statistically significant difference from the control group at $p< 0.05$. P values were calculated using an independent samples t-test

Table 3. Evaluated biochemical parameters in the study groups

Parameter	Reference range	Control (N=30)	Infected (N=30)	P value
ALT (IU/L)	10-42	22.7±4.3	22.6±4.7	NS
AST (IU/L)	10-40	22.8±3.2	23.5±3.8	NS
Alb. (g/dl)	3.5-5.0	4.2±0.9	4.3±0.29	NS
TP (g/dL)	6.6-8.3	7.5±0.3	7.5±0.3	NS
ALP (IU/L)	40-150	88.0±21.9	83.0±21.8	NS
GGT (IU/L)	10-49	20.0±5.1	20.9±5.8	NS
LDH (IU/L)	240-480	332±57	333±47	NS
Creat. (mg/dL)	0.5-1.5	1.01±0.07	1.03±0.13	NS
Na (mmol/l)	135-148	140.4±3.3	139.8±2.5	NS
K (mmol/l)	3.5-5.3	4.13±0.24	4.20±0.30	NS
p H	7.31-7.41	7.37±0.04	7.38±0.04	NS
Ca (mg/dl)	8.4 - 10.2	9.45±0.23	9.16±0.53	0.009*
Ca++ (mg/dl)	4.50-5.60	4.52±0.23	4.35±0.44	NS
Cl (mmol/l)	98-107	104.6±2.2	105.5±2.3	NS
Mg (mg/dl)	1.6-2.6	2.20±0.20	2.14±0.21	NS
Iron (ug/dl)	31-144	85.9±24.48	66.1±25.1	0.003*
MDA (IU/L)	--------	8.96±2.00	11.37±4.09	0.006*
GST (IU/L)	--------	383.3±250.9	338.3±236.7	NS

ALT (Alanine aminotransferase), AST (Aspartate aminotransferase), TP (Total protein), Alb (Albumin), ALP (Alkaline phosphatase), GGT (Gamma-glutamyl transferase), LDH (Lactate dehydrogenase), Creat. (Creatinine), Mg (Magnesium), Ca (Total calcium), Ca++ (Ionized calcium), Na (Sodium), K (potassium), Cl (Chloride), MDA (Malondialdehyde), GST (Glutathione-S-transferase). *Statistically significant difference from the control group at $p< 0.05$. P values were calculated using an independent samples t-test.

Table 4. GSTM1, GSTT1 genotypes and combined genotypic distribution among study groups.

Genotypes	Control (n = 30)	Infected (n = 30)	P- value
GSTM1 Positive	14 (46.7 %)	14 (46.7 %)	NS
GSTM1 Null	16 (53.3 %)	16 (53.3 %)	NS
GSTT1 Positive	15 (50 %)	15 (50 %)	NS
GSTT1 Null	15 (50 %)	15 (50 %)	NS
Positive M1/Positive T1	8 (26.7 %)	7 (23.3 %)	NS
Positive M1/Null T1	6 (20.0 %)	6 (20.0 %)	NS
Null M1/Positive T1	7 (23.3 %)	8 (26.0 %)	NS
Null M1/Null T1	9 (30.0 %)	9 (30.0 %)	NS
Data represented as (number, %), n=number, NS= non significant, n= number			

Table 5. GSTM1, GSTT1 genotypes and combined genotypic distribution in whole study population.

Genotypes	**Frequency (n, %)**	**P value**
GSTM1 Positive	28 (46.7 %)	NS
GSTM1 Null	32 (53.3 %)	
GSTT1 Positive	30 (50.0 %)	NS
GSTT1 Null	30 (50.0 %)	
Positive M1/Positive T1	15 (25%)	NS
Positive M1/Null T1	12 (20 %)	
Null M1/Positive T1	15 (25 %)	
Null M1/Null T1	18 (30 %)	
Data represented as (number, %), n=number, NS= non significant		

Table 6. Association between altered parameters and GSTM1 gene polymorphism

Group	Control		Infected		P- value
GSTM1	**M1 (Positive) A (N=14)**	**M1 (Null) B (N=16)**	**M1 (Positive) C (N=14)**	**M1 (Null) D (N=16)**	
Hemoglobin (g/dL)	14.46±0.7	14.61±0.9	14.22±0.86	14.03±0.74	NS
Hematocrit (%)	42.07±2.23	42.70±3.46	41.15±3.06	40.75±2.00	NS
MCV (fL)	84.67±3.68	83.19±4.40	80.79±5.09	82.37±4.67	NS
Monocytes (x103/µL)	0.31±0.09	0.34±0.13	0.38±0.10	0.48±0.12	0.01^{a}, 0.02^{b}
Eosinophils (x103/µL)	0.16±0.05	0.18±0.07	0.20±0.05	0.25±0.09	0.01^{a},0.02^{b}
Basophils (x103/µL)	0.03±0.01	0.04±0.01	0.04±0.01	0.05±0.02	0.02^{a},0.03^{b}
Calcium (mg/dl)	9.42±0.22	9.48±0.25	9.15±0.43	9.16±0.62	NS
Iron (ug/dl)	86.21±25.54	85.63±25.00	60.64±17.53	70.94±30.37	NS
MDA (IU/L)	8.60±2.29	9.27±1.73	9.99±3.59	12.57±4.23	0.01^{a},0.02^{b}
GST (IU/L)	514.9±277.1	268.3±157.7	351.7±307.7	326.6±161.2	0.026^{c}
Data represented as mean ± standard deviation, MCV (mean corpuscular volume), MDA (Malondialdehyde), GST (Glutathione-S-transferase). NS (non-significant), N (number), A (control with positive GSTM1), B (control with null GSTM1), C (infected with positive GSTM1), D (infected with null GSTM1), a (comparison between group A and D), b (comparison between group B and D), c (comparison between group A and B)					

Table 7. Association between altered parameters and GSTT1 gene polymorphisms.

Group	**Control**		**Infected**		**P- value**
GSTM1	**M1 (Positive) A (N=14)**	**M1 (Null) B (N=16)**	**M1 (Positive) C (N=14)**	**M1 (Null) D (N=16)**	
Hemoglobin (g/dL)	14.33±0.81	14.73±0.79	14.03±0.85	14.06±0.80	NS
Hematocrit (%)	42.10±2.25	42.72±3.53	40.79±2.69	41.67±2.80	NS
MCV (fL)	83.87±3.54	83.89±4.70	80.93±4.23	82.37±4.66	NS
Monocytes (x103/µL)	0.32±0.14	0.33±0.09	0.41±0.13	0.45±0.17	0.042[a]
Eosinophils (x103/µL)	0.17±0.07	0.18±0.04	0.21±0.07	0.24±0.09	0.030[a]
Basophils (x103/µL)	0.04±0.02	0.04±0.01	0.04±0.02	0.05±0.02	0.045[b]
Calcium (mg/dl)	9.44±0.18	9.46±0.28	9.22±0.48	9.10±0.59	NS
Iron (ug/dl)	87.20±29.15	84.60±20.55	64.60±21.32	75.66±28.02	NS
MDA (IU/L)	8.70±2.26	9.21±1.76	10.07±3.79	12.64±4.08	0.01[a]0.02[b]
GST (IU/L)	527.8±243.6	238.9±157.7	391.9±298.1	284.8±145.1	0.02[a]0.01[c]

Data represented as mean ± standard deviation, MCV (mean corpuscular volume), MDA (Malondialdehyde), GST (Glutathione-S-transferase). NS (non-significant), N (number), A (control with positive GSTT1), B (control with null GSTT1), C (infected with positive GSTT1), D (infected with null GSTT1), a (comparison between group A and D), b (comparison between group B and D), c (comparison between group A and B)

Discussion

Building on the established knowledge that genetic variations significantly influence susceptibility to infections by impacting how the body reacts, including inflammation regulation, tissue repair, and detoxification processes [24], this study investigated the relation between GST (M1 and T1) gene polymorphisms and hematobiochemical changes in *H. pylori*-infected patients.

Our findings align with previous research [25-29], demonstrating significant reductions in hemoglobin, hematocrit, mean corpuscular volume, serum iron, and total calcium in individuals infected with *H. pylori*. This agrees with the known effects of *H. pylori* infection, which can cause chronic inflammation and disrupt the gastric mucosal lining leading to increased acid secretion, hindering the body's ability to absorb essential nutrients like iron and calcium [30-32]. The human body tightly regulates iron stores to prevent both bacterial growth and oxidative damage [33]. This regulation can lead to iron deficiency when absorption is impaired, manifesting as decreased hemoglobin levels and reduced mean corpuscular volume [30, 34].

However, our findings also revealed a significant increase in monocytes, eosinophils, and basophils among *H. pylori*-infected individuals compared to the uninfected control group. While these observations align with some prior studies [35-37], they contradict the results reported in others [38,39]. This discrepancy might be explained by the chronic inflammatory response caused by persistent *H. pylori* infection, which is known to influence white blood cell counts in the bloodstream [40].

Malondialdehyde (MDA) as lipid peroxidation product levels were significantly higher in the *H. pylori*-infected group, consistent with previous studies [25, 41–43]. As an oxidative stress indicator, elevated levels of MDA in *H. pylori*-infected patients may be due to inflammation, immune responses, and tissue damage [44,45].

Glutathione-s-transferase (GST) enzyme activity was lower in the infected group, but the difference was not statistically significant. GST, an enzymatic antioxidant, may be depleted by the action of inflammation and oxidative stress brought on *H. pylori* infection [46,47], but MDA may also be produced in excess to protect the body from the inflammatory damage [48].

Our analysis of GSTM1 and GSTT1 genes showed equal distribution of both genotypes in control and *H. pylori*-infected groups. Roughly half the individuals in each group were null genotypes carriers for each gene (53.3% GSTM1-null, 50% GSTT1-null). Our findings partially align with previous research, demonstrating agreement in some aspects but also revealing discrepancies in others. In Italy, the prevalence of null variants is 49.2% for GSTM1 and 28.3% for GSTT1. Similarly, Spain shows a prevalence of 55.3% for null GSTM1 and 27.7% for null GSTT1. In Cameroon, the prevalence of null variants is 27.8% for GSTM1 and 46.8% for GSTT1. In Ethiopia, the prevalence of null variants is 43.8% for GSTM1 and 37.3% for GSTT1 [49]. A study among Egyptians

found that the prevalence of null variants is 55.5% for GSTM1 and 29.5% for GSTT1 [53], while other study reported that prevalence of null variants is 76% for GSTM1 and 56.5% for GSTT1 [54]. Other research reported a notable high prevalence of the GSTM1 positive genotype (59.25%) compared to the GSTM1 null genotype (40.75%) and marked significant variation in the frequencies of the GSTT1 positive (68.00%) and GSTT1 null (32.00%) genotypes among Egyptians [55].

Further analysis stratified by genotypes revealed interesting patterns. Among *H. pylori*-infected individuals, those with both GSTM1-null and GSTT1-null genotypes displayed significantly higher levels of monocytes, eosinophils, basophils, and MDA, alongside lower glutathione-S-transferase (GST) activity compared to control groups. This suggests a potential association between null genotypes and these markers. Additionally, within the control group, individuals with null genotypes for both GSTM1 and GSTT1 exhibited significantly lower GST enzyme activity compared to those with positive genotypes for both enzymes. This finding highlights the potential influence of these genotypes on baseline GST activity.

Our findings suggest an association between genetic variations in GSTM1/GSTT1 and hematobiochemical alterations linked to *H. pylori* infection. To our knowledge, no previous studies have explicitly reported this specific relationship. Elkhalifa and others in 2021 reported that infection with *H. pylori* may be involved in numerous additional gastric disorders, including hematobiochemical changes [39]. In 2021, Tamer and others found that GST (M1, T1) genes modulate GST enzyme activity, and individuals with null genotypes have lower detoxification ability, which in turn increases susceptibility to diseases [56].

The association between deletion variants of the GSTM1/GSTT1 genes, which cause decreased GST enzyme activity, and oxidative stress related diseases has been reported in several previous studies [57–61]. Since GST enzymes play a crucial role in detoxification, individuals with null genotypes for these polymorphisms may have a reduced capacity to eliminate xenobiotics and reactive oxygen species (ROS) generated by *H. pylori* infection. This impaired detoxification could potentially contribute to the observed alterations [62]. Thus, it looks logical to find more prominent hematological and biochemical alterations among GSTM1 and GSTT1 null genotypes carriers in our studied *H. pylori*-infected patients. Finally, the study provides a valuable starting point for future research on the impact of GSTM1/GSTT1 gene polymorphism in hematobiochemical alterations among *H. pylori*-infected patients. Our findings could contribute to identifying individuals with specific GST genotypes who might be more susceptible to developing hematobiochemical changes or other complications upon *H. pylori* infection. This information could be used to develop personalized risk stratification strategies for *H. pylori* testing and management. Larger clinical trials are needed to validate the clinical significance of these findings and assess the potential benefits of personalized treatment approaches based on GST genotypes. Additionally, exploring the influence of *H. pylori* and other genetic variations could provide a more comprehensive picture of this complex interplay. Finally, this study lays the groundwork for future investigations exploring the influence of other gene polymorphisms and diverse parameters on gene-susceptibility interactions.

Conclusion

The present study indicated that *H. pylori* infection can cause hematological and biochemical alterations, which may be modulated by GSTM1/GSTT1 genes polymorphism, with a higher susceptibility in null genotypes carriers. While these findings offer valuable insights, the generalizability of our results may be limited by the study population, which was drawn from a single geographic region. To comprehensively elucidate the intricate relationship between genetic polymorphisms, hematobiochemical parameters, and disease outcomes, larger-scale studies investigating diverse populations and a broader spectrum of variables are warranted.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Institutional Review Board of the Faculty of Medicine at Zagazig University on August 20, 2023 (protocol code ZU-IRB#:101034-20-8-2023).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used in this study are available upon request through the corresponding author.

Competing interests

The authors declare that they have no competing interests.

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