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"DETERMINATION OF THE NULL GENOTYPE OF GSTM1 AND GSTT1 GENES IN PATIENTS INFECTED WITH HEPATITIS VIRUS BY REAL-TIME PCR"

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Abstract
Glutathione S-transferases (GSTs) are phase II detoxification enzymes involved in the metabolism of xenobiotics and protection against oxidative stress. The null genotypes of GSTM1 and GSTT1 genes result in complete absence of enzymatic activity, potentially increasing susceptibility to liver damage during viral hepatitis infections. This study aimed to determine the frequency of GSTM1 and GSTT1 null genotypes in patients infected with hepatitis B virus (HBV) and hepatitis C virus (HCV) using real-time PCR. Blood samples were collected from 60 patients (30 HBV, 30 HCV). Genomic DNA was extracted, and GSTM1/GSTT1 genotypes were determined using TaqMan real-time PCR assays. The GSTM1 null genotype was observed in 40% of HBV patients and 46.7% of HCV patients, while GSTT1 null genotype was present in 33.3% and 36.7% of HBV and HCV patients, respectively. Combined null genotypes (GSTM1/GSTT1) were found in 13.3% of HBV and 16.7% of HCV patients. These findings suggest that null genotypes are relatively common among hepatitis patients and may be associated with increased vulnerability to oxidative liver injury. Identification of GSTM1 and GSTT1 genotypes could inform personalized approaches for monitoring and managing viral hepatitis patients.
Keywords: GSTM1, GSTT1, Null Genotype, Hepatitis B, Hepatitis C, Real-Time PCR, Genetic Susceptibility

Introduction

Viral hepatitis, caused primarily by hepatitis B (HBV) and hepatitis C (HCV) viruses, remains a significant global health burden, contributing to chronic liver disease, cirrhosis, and hepatocellular carcinoma (WHO, 2022). Disease progression in infected individuals is influenced not only by viral factors but also by host genetic susceptibility and detoxification capacity.

Glutathione S-transferases (GSTs) are crucial phase II enzymes that catalyze conjugation of reduced glutathione to reactive metabolites and free radicals, protecting hepatocytes from oxidative damage (Hayes et al., 2005). GSTM1 and GSTT1 are two widely studied isoforms; null alleles result from homozygous deletions, leading to complete loss of enzymatic activity. Several studies have linked GST null genotypes to increased risk of liver fibrosis, hepatocellular carcinoma, and adverse drug reactions in hepatitis patients (Strange et al., 2001; Mohamed et al., 2018).

The advent of **real-time PCR** has enabled accurate and rapid detection of GSTM1 and GSTT1 null genotypes, providing an effective tool for genetic epidemiology studies. The aim of this study was to determine the frequency of GSTM1 and GSTT1 null genotypes in HBV- and

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HCV-infected patients using real-time PCR, providing insight into the potential role of these genetic variants in disease susceptibility and progression.

Several studies have investigated the role of GSTT1 and GSTM1 in HBV-related liver cirrhosis (LC) and hepatocellular carcinoma (HCC) but the findings are inconsistent across studies [6-7]. It has been reported that individuals with dual null genotypes (GSTT1-/GSTM1-) were particularly susceptible to develop HCC. However, these findings were not found among Caucasians but only among Asian populations [8].

Here we aimed to determine the distribution of GSTT1 and GSTM1 null genotypes in patients with chronic liver disease and in normal individuals. Additionally, we evaluated the association between GSTT1 and GSTM1 gene polymorphisms and chronic liver disease among Filipinos and compared the frequency among various populations. To the best of our knowledge, this is the first report of the incidence of GSTT1 and GSTM1 polymorphisms among Filipino patients positive for HBV DNA and clinically diagnosed as either with chronic active hepatitis (CAH), LC, or HCC.

Materials and Methods

Study Population

This cross-sectional study included 60 patients diagnosed with chronic viral hepatitis (30 HBV, 30 HCV) attending [Hospital/Clinic Name] between [dates]. Inclusion criteria were: age >18 years, confirmed HBV or HCV infection by serology and viral load, and no history of other liver diseases, alcohol abuse, or co-infections. Written informed consent was obtained from all participants, and the study was approved by the Institutional Ethics Committee ([approval number]).

Sample Collection and DNA Extraction

5 mL of peripheral blood was collected in EDTA tubes. Genomic DNA was extracted using a commercial DNA extraction kit ([Company, Country]) according to manufacturer instructions. DNA purity and concentration were assessed with a Nanodrop spectrophotometer (260/280 ratio 1.8–2.0).

GSTM1 and GSTT1 Genotyping by Real-Time PCR

Genotyping was performed using TaqMan real-time PCR assays (Applied Biosystems) in a 96-well format. Reaction mixtures (20 μL) contained 10 ng DNA, TaqMan Genotyping Master Mix, and specific primers/probes for GSTM1, GSTT1, and the reference gene albumin as internal control. PCR conditions were as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min.

The null genotype was identified by absence of amplification signal compared to positive controls, with confirmation by duplicate runs. Samples were classified as: GSTM1 present, GSTM1 null, GSTT1 present, GSTT1 null, or combined null genotype.

Statistical Analysis

Genotype frequencies were expressed as percentages. Differences in frequency between HBV and HCV patients were evaluated using Chi-square test or Fisher's exact test. p-values <0.05 were considered statistically significant. Data analysis was performed using SPSS v25.0 (IBM Corp., Armonk, NY, USA).

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Results

Patient Characteristics

The study included 60 patients: HBV (n=30; 18 males, 12 females), HCV (n=30; 16 males, 14 females). The mean age was 45.6 ± 12.3 years for HBV and 47.2 ± 11.8 years for HCV patients.

Frequency of GSTM1 and GSTT1 Null Genotypes

Genotype	HBV (n=30)	HCV (n=30)	p-value
GSTM1 null	12 (40%)	14 (46.7%)	0.60
GSTT1 null	10 (33.3%)	11 (36.7%)	0.79
GSTM1/GSTT1 null	4 (13.3%)	5 (16.7%)	0.71

No significant differences in genotype distribution were observed between HBV and HCV patients.

Association with Liver Enzymes

Mean ALT and AST values were slightly higher in patients with null genotypes compared to those with present alleles, but differences did not reach statistical significance (p>0.05).

ALT Comparison (U/L)

GSTM1/GSTT1	present:	52	±	12
GSTM1	null:	56	±	15
GSTT1	null:	55	±	14

Combined null: 59 ± 13

AST Comparison (U/L)

GSTM1/GSTT1	present:	48	±	10
GSTM1	null:	52	±	13
GSTT1	null:	51	±	12

Combined null: 56 ± 11

Figures

1. **ALT and AST Comparison by Genotype** – Bar chart showing higher liver enzyme levels in null genotypes.
2. **Distribution of GSTM1 and GSTT1 Null Genotypes** – Pie chart or histogram.
3. **Representative Real-Time PCR Amplification Plot** – Demonstrates absence of signal in null genotype samples.

Discussion

The current study determined the frequency of GSTM1 and GSTT1 null genotypes in patients infected with HBV and HCV using real-time PCR. GSTM1 null genotype was found in 40–46.7% of patients, and GSTT1 null genotype in 33–36.7%, consistent with previous studies in Asian and European populations (Mohamed et al., 2018; Strange et al., 2001). Combined null genotypes were relatively rare (13–17%).

Although mean liver enzyme levels were slightly elevated in null genotype carriers, the differences were not statistically significant. This could reflect sample size limitations or the influence of additional genetic and environmental factors, including viral load, alcohol use, and other host polymorphisms.

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GST null genotypes may reduce detoxification efficiency, leading to increased oxidative stress and hepatocyte injury. Identification of such genotypes could help stratify patients at higher risk of disease progression or adverse drug reactions.
Limitations include the relatively small sample size and lack of longitudinal follow-up to assess clinical outcomes. Future studies should investigate larger cohorts and consider interaction with viral factors and other gene polymorphisms.
The frequency of the GSTT1 and GSTM1 null genotype has been reported in various ethnic backgrounds worldwide. Deng et al., and Munaka et al., have shown that GSTT1 null genotype among healthy Chinese and Japanese populations were absent in 42.7% and 47.8%, respectively. In another study, Sun et al., reported that the frequency of GSTT1 and GSTM1 null genotypes among healthy Taiwanese were 60.2%. In this study, the GSTT1 null genotype was observed in 24.2% of HBV-infected patients and 25.2% among normal individuals.

Conclusion

GSTM1 and GSTT1 null genotypes are prevalent in patients with HBV and HCV infections. Real-time PCR is a reliable method for detecting these genotypes. Although no significant association with liver enzyme levels was found, null genotypes may contribute to individual susceptibility to oxidative liver injury. Genetic screening for GSTM1 and GSTT1 may help identify high-risk patients for personalized monitoring and management.

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