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Frequency of Glutathione S-Transferase Polymorphisms in Patients with Metabolic Dysfunction Associated Steatotic Liver Disease (MASLD) in North Coastal Andhra Pradesh a Case-Control Study

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KEYWORDS

MASLD, NAFLD, Glutathione S Transferase, GSTM1, GSTT1, GSTP1, Fatty Liver, Andhra Pradesh.

ABSTRACT:

Background and Aim: Glutathione S-transferase (GST) enzymes have critical role in detoxification and oxidative stress management, which may influence metabolic dysfunction associated steatotic liver disease (MASLD) development. Genetic polymorphisms of GSTs might play a role in the pathogenesis and could impact inter individual susceptibility to MASLD. Aim of this study investigates the relationship between MASLD susceptibility and GSTT1, GSTM1 and GSTP1 gene polymorphisms in a cohort from North Coastal Andhra Pradesh.

Methods: A total of 300 individuals, 150 clinically documented MASLD patients (93 Male; Mean age: 42.89_11.31 years), and 150 healthy controls (67 Male; Mean age: 40.07_10.43) were included in the study. DNA was isolated from blood using salting out method. The genotypes of GSTM1 and GSTT1 were determined by multiplex polymerase chain reaction (PCR) and that of GSTP1 by tetra-primer Amplification Refractory Mutation System PCR. Quality control measures were implemented to ensure genotyping accuracy and reliability. The frequencies of GSTM1, T1 and P1 genotypes in MASLD cases and controls were compared.

Results: In the present study significant difference was detected between the MASLD group and control group for GSTM1 (OR=0.2677, 95% CI=0.1413-0.5071, p =<0.0001) and GSTT1 (OR=0.5301, 95% CI=0.3215-0.8738, p =0.0128) polymorphisms. The frequency of GSTP1 Ile/ Val and Val/Val genotypes was significantly higher in MASLD group (41.3% and 15.3%) than in the control group (17.3% and 4.6%) and may be a risk factor for susceptibility to MASLD (OR=3.3601, 95% CI=1.9714-5.7272, p =<0.0001 for Ile/Val; OR=3.6997, 95% CI=1.5358-8.9121, p =0.0035 for Val/Val).

Conclusion: GSTM1, GSTT1 and GSTP1 polymorphisms were seen significantly more frequently in our cohort of MASLD patients than in healthy controls. This study concludes that GSTM1, GSTT1 and GSTP1 polymorphisms may be associated with increased susceptibility to MASLD

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INTRODUCTION:

Metabolic dysfunction associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD), is currently a global health concern due to its growing incidence and association to metabolic disorders such obesity, insulin resistance, and dyslipidemia (1,2). MASLD comprises a wide range of liver conditions, particularly simple steatosis, non-alcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular cancer (3). Despite significant research, the precise etiology of MASLD remains unknown, with both environmental as well as genetic factors influencing its pathogenesis.

Glutathione S-transferases (GSTs) enzymes are a group of phase II detoxification enzymes that synthesize glutathione with electrophilic compounds (4), enabling their excretion and neutralization (5). GST enzymes also have significant functions in regulating oxidative stress and inflammation, both of which are vital to MASLD pathogenesis (6). Genetic polymorphisms in GST genes, such as GSTM1, GSTT1, and GSTP1, have been linked to altered activity of enzymes and susceptibility to a variety of conditions, including liver disorders (7, 8).

GSTM1 & GSTT1

The deletion variations linked to the GSTM1 and GSTT1 genes are found at chromosome 1 1p13.3 and 22q11.23, respectively, according to earlier research. The homologous protein is completely devoid of enzymatic activity in individuals with deletion variants (null/null) of the GSTM1 or GSTT1 genes (9, 10, 11). One's capacity to detoxify genotoxic substances is impacted when a structural deletion (Null mutation) results in a loss of function in the GST classes M1 and T1 (12, 13). DNA hydroperoxides and alkyl halide, a component of cigarette smoke, are examples of polycyclic aromatic hydrocarbon diol epoxides that GSTM1 catalyzes and detoxifies, whereas GSTT1 catalyzes and detoxifies benzo (a) pyrene diol epoxide and acrolein (13, 14). Deficits in GSTM1 and GSTT1, either separately or in combination, are believed to have diminished detoxifying properties, thus have a major role in raising the risk of developing several kinds of cancer.

The GSTM1 and GSTT1 genes encode the GST Mu-1 and Theta-1 enzymes, respectively. Relatively often, GSTM1 and GSTT1 have complete gene deletions (loss of functioning gene copy). While >22% and >14% of Asians and >27% and >37% of Africans are homozygous for GSTM1 and GSTT1 gene deletions, respectively, about 50% and 30% of Caucasians are homozygous for these genes (15).

GSTP1

A member of several major enzyme families, glutathione S-transferase P1 is encoded by the GSTP1 gene (located at 11q13.2) and is crucial to the antioxidant and detoxification systems. The GSTP1 gene's rs1695 A/G polymorphism results in the Ile105Val amino acid alteration, which can significantly modify GSTP1 activity. (16)

GST pathways interacting with MASLD

MASLD has the characteristics by complicated interactions between genetic predisposition, environmental variables, and multiple cellular pathways. GSTs are enzymes that catalyze the conjugation of glutathione to electrophilic molecules, allowing them to be eliminated from the body more efficiently. While GSTs are predominantly involved in xenobiotic metabolism, they also regulate oxidative stress, inflammatory conditions, and proliferating cells, all of which play important roles in the onset of MASLD (17).

Here's how GST pathways interact with MASLD genes. GSTs, particularly GSTM1, GSTT1, and GSTP1, are involved in the detoxification of endogenous and exogenous compounds, including reactive oxygen species (ROS), lipid peroxides, and environmental toxins (18). GST gene variations can influence enzymatic activity and efficacy in eliminating hazardous chemicals. Individuals with GSTM1 and GSTT1 null variations (deletions) lose catalytic activity of enzymes, making detoxification mechanisms less efficient (19).

GST polymorphisms may hinder detoxification, resulting in risky intermediate accumulation and oxidative stress (20). This may lead to hepatocyte injury and increase the risk of MASLD. GSTs contribute to preventing the effects of oxidative stress by eliminating ROS and lipid peroxides. GSTP1, in particular, promotes to protect cells from oxidative damage (21).

GST gene variations may influence the cell's ability to combat oxidative stress and its susceptibility to liver injury triggered by oxidative stress. Dysregulated oxidative stress and inflammation are important drivers

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of MASLD (Metabolic Associated Steatotic Liver Disease) development, causing hepatocyte damage and inflammation. GSTs (Glutathione S-transferases) are primarily recognized for their role in detoxification, but emerging evidence suggests they also play a crucial role in lipid metabolism, which is vital for maintaining hepatic function. Variations in GST genes may alter the efficiency of antioxidant defenses, thereby increasing oxidative damage and inflammation, contributing to the progression of liver diseases like MASLD. GSTP1 has been connected to lipid peroxidation and homeostasis regulation. GSTP1 gene variations may alter lipid metabolism and lead to dyslipidemia, a prevalent sign of MASLD (22).

MASLD pathogenesis is characterized by disrupted lipid metabolism, which includes increased hepatic lipid buildup (23, 24) and abnormal lipid droplet dynamics. GSTs may indirectly affect insulin signaling and lipogenesis through oxidative stress and inflammation (25). MASLD development is primarily driven by oxidative stress-induced insulin resistance (26) and dysregulated lipogenesis. Genetic differences in GST genes may worsen these processes, increasing MASLD susceptibility and severity.

In brief, variations in genes in GST pathways can influence MASLD risk by affecting detoxifying capability, oxidative stress response, inflammation, lipid metabolism, and insulin signaling (27). Understanding the link between GST genetics and MASLD pathophysiology may shed light on disease mechanisms and potential therapeutic targets for intervention (28, 29).

This study aims to investigate the association between GSTM1, GSTI1, and GSTP1 gene polymorphisms and susceptibility to MASLD in the population of the Visakhapatnam region. Understanding the genetic origins of MASLD may provide insights into its pathophysiology, aid in risk assessment, and contribute to individualized methods of treatment.

MATERIALS AND METHODS

Study subjects

The study comprised 300 people, mostly from the Visakhapatnam region, including 150 MASLD cases (93 males, 57 females) and 150 controls (67 males, 83 females) who had no history of MASLD, with their distributions shown in Graphs 1 and 2 respectively. Blood samples were collected and delivered to the Department of Human Genetics at Andhra University in Visakhapatnam for analysis. All subjects gave prior informed consent, and relevant clinical and demographic information was obtained.

Cases:

Inclusion criteria:

✓ MASLD patients over 18 years
✓ MASLD patients who had other comorbidities were also included.

Exclusion criteria:

✓ Patients unwilling to provide samples.
✓ Diagnosed pregnant women and cases under the age of 18 yrs. were excluded.

Controls:

Inclusion criteria

✓ Subjects without MASLD and no family history of the condition were included.

Exclusion criteria:

✓ Individuals who are unwilling to provide samples.
✓ Pregnant women and individuals under the age of 18 were omitted.

The demographic data of the subjects is compiled in Table 1, with the mean, standard deviation, and median values representing the subjects' ages.

Table 1. Demographic characteristics of subjects

Characteristics	No. of cases	No. of controls
Sex		
Male	**93**	**67**
Female	**57**	**83**
Age (yrs)		
Mean	**42.89±11.31**	**40.07±10.43**
Median	**41**	**39**

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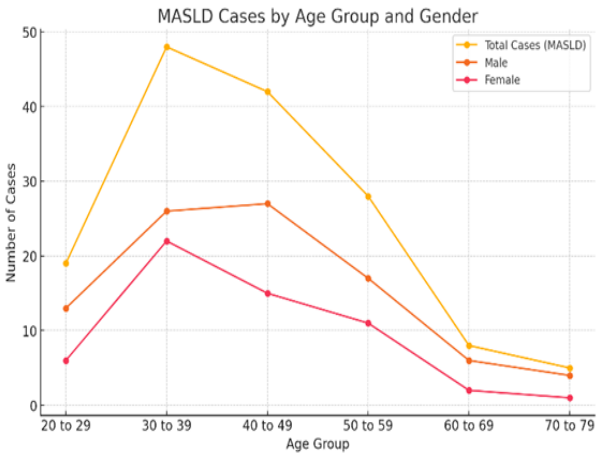
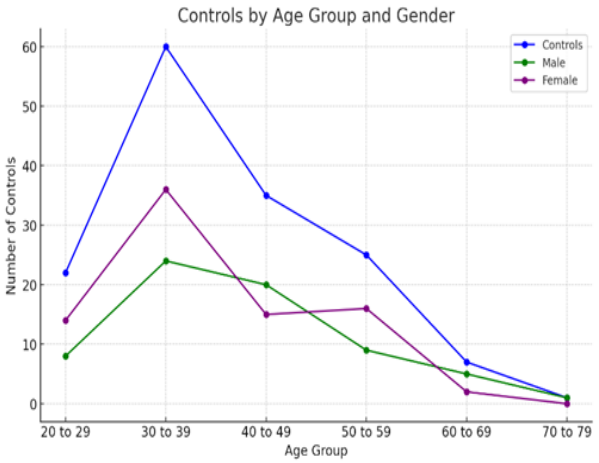
Age (yrs): Specifies the individuals' age in years. Mean: Shows the range or variety in ages around the mean by displaying the subjects' average age and standard deviation. The term "median" refers to the average age of the subjects, or the age at which half of them are younger and the other half older.



Graph 1 The graph depicts the distribution of MASLD cases by age and gender. Total MASLD cases is represented by the yellow line. Male is represented by an orange line. Females are represented by a red line.



Graph 2 The graph displays the number of controls by age and gender. Total controls are represented by a blue line. Male is represented by a green line. Females are represented by a purple line.

GENOTYPING

Genomic DNA was extracted from peripheral blood samples using salting out method (30). Quality and quantity analysis of DNA was performed using Gel electrophoresis and Spectrophotometer methods. The GSTM1 and GSTT1 null polymorphisms were genotyped using multiplex PCR method with Toll-like receptor 2 (TLR2) primers as the internal control. The primers used for GSTM1 genotyping were forward 5' GCTGCCCTACTTGATTGATG 3' and reverse 5' CCCCAAATCCAAACTCTGTC 3', which produced a fragment of 325 bp. The primers used for GSTT1 genotyping were forward 5' TTCTGCTTTATGGTGGGGTC 3' and reverse 5' GTGATGTTCCCTGTTTTCCT 3' which produced a fragment of 542 bp. The 259 bp TLR2 gene was amplified by using forward 5' GATGCATTTGTTTCTTACAGTGAGCG 3' and reverse 5' TCTCATCAAAAAGACGGAAATGGG 3'(25). The multiplex PCR was performed under the following conditions: initial denaturation at 95°C for 5 min, followed by 25 cycles of denaturation at 95 °C for 30 sec, annealing at 60 °C for 25 sec, extension at 72 °C for 30 sec and final extension at 72 °C for 10 min. PCR products were analyzed by electrophoresis on a 2% agarose gel and visualized by ethidium bromide staining (Figure 1, 2).

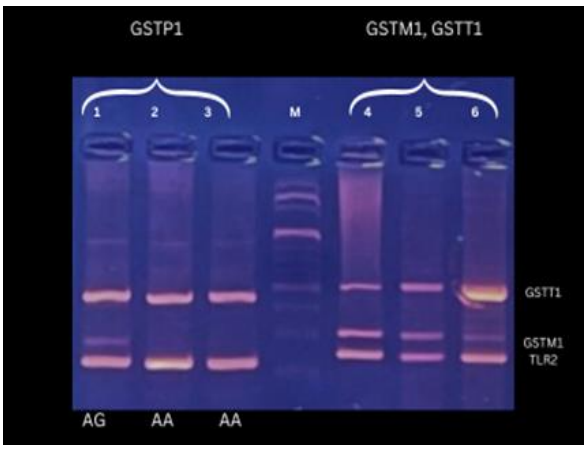


Figure 1Gel image representing the distribution of amplified GSTP1, GSTM1 and GSTT1 gene fragments: Lane 1-AG genotype (233bp, 290bp, 467bp), Lane 2-AA genotype (233bp, 467bp), Lane 3-AA genotype (233bp, 467bp), M- is the molecular weight marker, Lane 4-present/present (259bp, 325bp, 542bp), Lane 5-present/present (259bp, 325bp, 542bp), Lane 6-present/ present (259bp, 325bp, 542bp).

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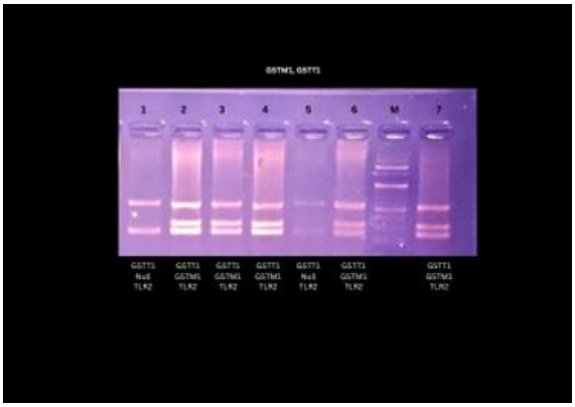

Figure 2 Gel image representing the distribution of amplified GSTM1 and GSTT1 gene fragments: Lane 1 GSTT1 present/null (259bp, 542bp), Lane 2 GSTT1/GSTM1 present/present (259bp, 325bp, 542bp), Lane 3 GSTT1/GSTM1 present/present (259bp, 325bp, 542bp), Lane 4 GSTT1/GSTM1 present/present (259bp, 325bp, 542bp), Lane 5 GSTT1 present/null (259bp, 542bp), M- is the molecular weight marker, Lane 7 GSTT1/GSTM1 present/present (259bp, 325bp, 542bp).

The GSTP1 gene polymorphism was analyzed using the tetra-primer Amplification Refractory Mutation System (ARMS) PCR. GSTP1 primers (467bp), located in Exon 5, targeting the 105 Ile/Val polymorphism (SNP: rs1695). External primers: Forward outer 5'CAGGTGTCAGGTGAGCTCTGAGCACC3', reverse outer 5' ATAAGGGTGCAGGTTGTGTCTTGTCCCA 3'. Internal primers: 233bp A allele/ 290bp G allele Forward inner (A allele or Ile allele): 5' CGTGGAGGACCTCCGCTGCAAATCCA 3' Reverse inner (G allele or Val allele): 5' GCTCACATAGTTGGTGTAGATGAGGGATAC 3' (30). The PCR protocol was initial denaturation at 95°C for 5 min, followed by 25 cycles of denaturation at 95 °C for 30 sec, annealing at 65 °C for 25 sec, extension at 72 °C for 15 sec and final extension at 72 °C for 10 min. The amplified products were electrophoresed in 2% agarose gel (Figure 3)

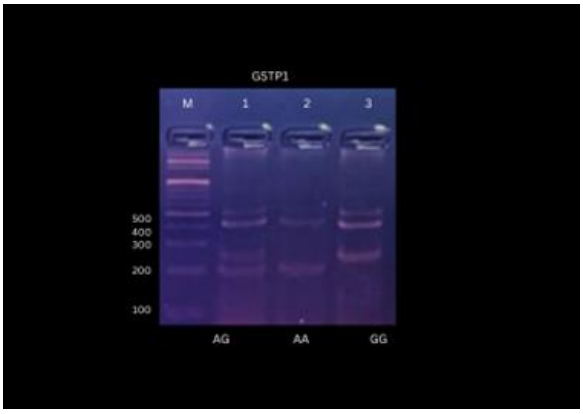

Figure3 Gel image representing the distribution of amplified GSTP1 gene fragments: M- is the molecular weight marker, Lane 1-AG Ile/Val (233bp, 290bp, 467bp) genotype, Lane 2-AA Ile/Ile (233bp, 467bp) genotype, Lane 3-GG Val/Val genotype (290bp, 467bp).

STATISTICAL ANALYSIS

Statistical analysis was conducted by using MedCalc statistical software version 22.023 to assess the association between GST gene polymorphisms and susceptibility to MASLD. Genotype frequencies were compared between MASLD cases and controls using Altman's Odd's ratio and 95% confidence intervals (CI). p values of less than 0.05 were considered statistically significant (31, 32).

RESULTS

Based on the provided results (Table 2), the genetic variations in GSTM1, GSTT1, and GSTP1 genes are associated with the prevalence of MASLD. Specifically, the presence of the GSTM1 present genotype, GSTT1 present genotype, GSTP1 Ile/Ile genotype, and Ile allele of GSTP1 are protective against MASLD, while certain variants of GSTP1 (Ile/Val and Val/Val genotypes) are associated with increased risk. These findings highlight the potential role of GST gene polymorphisms in the pathogenesis of MASLD particularly in the Visakhapatnam region population.

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Table 2. Frequency distribution of GSTM1, GSTT1 and GSTP1 polymorphisms with MASLD risk

Frequency Distribution of the GSTM1, GSTT1, and GSTP1 Genotypes in subjects with and without Metabolic dysfunction associated steatotic liver disease (MASLD)				
GST Genotype	**MASLD n (%)**	**Control n (%)**	**OR (95% CI)**	**p-Value**
GSTM1				
Present	106(70.66)	135(90)	0.2677 (0.1413 to 0.5071)	0.0001
Null	44(29.33)	15(10)		
GSTT1				
Present	94(62.66)	114(76)	0.5301 (0.3215 to 0.8738)	0.0128
Null	56(37.33)	36(24)		
GSTP1				
AA Ile/Ile	65(43.33)	117(78)	0.2157 (0.1304 to 0.3569)	< 0.0001
AG Ile/Val	62(41.33)	26(17.33)	3.3601 (1.9714 to 5.7272)	< 0.0001
GG Val/Val	23(15.33)	7(4.66)	3.6997 (1.5358 to 8.9121)	0.0035
GSTP1 alleles				
Ile allele	192(64)	260(86.66)	0.2735 (0.1819 to 0.4113)	< 0.0001
Val allele	108(36)	40(13.33)		

MASLD n (%): The number and percentage of patients with Metabolic Associated Steatotic Liver Disease (MASLD). Control n (%): The number and percentage of control subjects without MASLD. OR (95% CI): Odds ratio with a 95% confidence interval, representing the odds of having the genotype in MASLD patients compared to controls. The p-value indicates the statistical significance of the association between the genotype and MASLD.

DISCUSSION

The genetic polymorphisms in GSTM1, GSTT1, and GSTP1 are strongly linked to MASLD, according to this study's conclusive data. Furthermore, our results show that the susceptibility to MASLD is significantly increased in individuals with the GSTM1 (null genotype) and GSTT1 (null genotype polymorphisms. The odds ratio for GSTM1 was 0.2677 (95% CI=0.1413-0.5071, p=<0.0001), suggesting that individuals with the GSTM1 polymorphism experience a significant reduction in the risk of MASLD. Similarly, a modest protective effect was suggested by the odds ratio for GSTT1 of 0.5301 (95% CI=0.3215-0.8738, p=0.0128).

The study also emphasizes how the MASLD group had higher frequencies of GSTP1 Ile/Val and Val/Val genotypes than the control group. An odds ratio of 3.3601 (95% CI=1.9714-5.7272, p=<0.0001) indicated that 41.3% of the MASLD group and 17.3% of the control group had the GSTP1 Ile/Val genotype. 15.3% of the MASLD group and 4.6% of the control group had the GSTP1 Val/Val genotype, with an odds ratio of 3.6997 (95% CI=1.5358-8.9121, p=0.0035) for the former group. Based on these findings, there appears to be a considerable increased risk of MASLD development in those with the GSTP1 Ile/Val and Val/Val genotypes.

Numerous common polymorphisms exist in human GST genes, including the complete absence of the GSTM1 and GSTT1 genes, which can occur in up to 20%–50% of various groups and populations. The frequency of the

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null genotype for GSTT1 and GSTM1 varies among ethnic groups, with 80% of Asians and 20% of Caucasians having the GSTT1 deletion (33).

However, 38%–67% of Caucasians, 33%–63% of East Asians, and 22%–35% of Africans and African Americans have the GSTM1 null genotype (34).These results are in line with previous research indicating that genetic variations in the GST gene may affect a person's vulnerability to a number of liver illnesses, including MASLD (35). Genetic differences in the GST family of enzymes can affect the activity of these enzymes, which can affect the body's capacity to control oxidative stress and detoxify hazardous substances. The GST family of enzymes is essential to detoxification processes. The role that the GSTM1 and GSTT1 genes play in the conjugation and removal of harmful chemicals, which lowers inflammation and liver damage, may be the cause of the protective benefit linked to their existence.

Furthermore, the substantial correlation shown between GSTP1 polymorphisms and MASLD is consistent with earlier research showing that changes in the GSTP1 gene might impact the body's response to oxidative stress and enzyme activity. Reduced detoxifying ability may be the cause of the elevated risk seen in people with the GSTP1 Ile/Val and Val/Val genotypes. This could result in an accumulation of ROS and eventual liver damage.

Studies on the distribution frequency of allelic variations in the GSTP1 A313G polymorphism (36) suggest that the G allele is significantly more prevalent in patients with NAFLD than in healthy individuals in the Ukrainian population ($\chi^2 = 5.69$, $P = 0.017$). This information is consistent with the findings of Hashemi et al. (31), who showed that the GSTP1 gene's G allele is a risk factor for the development of NAFLD. According to an investigation (37), carriers of the A313G polymorphism in the GSTP1 gene with the GG genotype had higher total bilirubin levels in their blood compared to those with the AA and AG genotypes. Additionally, the presence of the G allele was associated with elevated alanine aminotransferase activity, which was significantly higher in patients with the AG and GG genotypes of NAFLD compared to those with the AA genotype.

Regarding the investigation of cirrhosis and pancreatitis susceptibility in alcoholics, Burim et al.'s findings (38) found that GSTP1 Val allele carriers were more likely to develop both conditions due to polymorphisms in the GST and cytochrome 450 genes. The Val/Val GSTP1 genotype was linked to cryptogenic cirrhosis by Ghobadloo et al. (39). This finding may be explained by the reduced detoxifying activity of the protein, which links this polymorphism to an increased chance of developing the disease.

According to research by Goncharova et al. (40), patients with liver cirrhosis who carry the AA genotype of the GSTP1 gene had a 2.5-fold greater survival rate than patients who have the GG and AG genotypes.

When compared to non-alcoholic or alcoholic controls, Khan et al. [41] demonstrated that individuals with the GSTM1 null genotype had a higher chance of developing alcoholic cirrhosis. Patients with a combination of GSTM1 and GSTT1 null genotypes were found to have a significantly increased risk of developing alcoholic liver cirrhosis (42), In prior research, the authors found that GSTs interacted with variant genotypes of either cytochrome P450 2E1, which produces free radicals, or manganese superoxide dismutase, which detoxifies free radicals, increasing the risk of alcoholic liver cirrhosis multiple times. Thus, conclusions can be drawn about the potential role of gene-gene interactions in regulating the development of alcoholic liver cirrhosis (42).

Overall, this work highlights the role of genetic variables in the etiology of MASLD. The identification of GSTM1, GSTT1, and GSTP1 polymorphisms as significant risk factors sheds light on the molecular mechanisms underlying MASLD and identifies potential treatment targets. Future studies should explore the functional implications of these polymorphisms, as well as the potential for personalized medicine approaches to managing MASLD based on individual genetic profiles.

DECLARATIONS

ETHICS APPROVAL: This is a Case-control study. Andhra University Institutional Ethical committee has reviewed and approved the study protocol (Approval No. IEC 34/06-01-2020).

CONSENT TO PARTICIPATE:

Informed Consent was obtained from all the participants included in the case-control study

CONSENT TO PUBLISH:

The authors affirm that human research participants provided informed consent for publication of the data.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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