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Research Article

Study on the association of Glutathione S-transferase Mu 1 *(GSTM1)* gene polymorphism with acute decompensated heart failure with reduced ejection fraction

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Abstract
The most frequent cause of hospitalization in adults over 65 in Western nations is acute decompensation of heart failure (ADHF). Due to the high death rate, it places a heavy load on healthcare systems as well as people. The present study's objective was to ascertain the connection between polymorphisms in the Glutathione S-transferase Mu 1 *(GSTM1)* gene and heart-failure-reduced ejection fraction (HFrEF) patients. Sixty patients with a mean age of 25–94 were taken from both genders after the clinical diagnosis by a specialist to cases who were referred to the Ibn Al-Baitar Specialized Center for Cardiac Surgery and Ramadi Teaching Hospital, and thirty apparently healthy people were taken as a control. Blood and serum containing EDTA were drawn from sick and healthy people to extract DNA for multiplex PCR detection of the GSTM1 polymorphism. The GSTM1 genotype was found in 23 (38.33%) and the null gene in 37 (61.66%) of the 60 ADHF patients. Of 30 healthy people, 8 (26.66%) had the GSTM1 gene and 22 (73.33%) had the null gene. According to the present study, the etiology of acute decompensated heart failure (ADHF) and the antioxidant null gene (GSTM1) are related. The present study has been achieved to determine the relationship between the presence of the antioxidant gene (GSTM1) and the incidence of ADHF with reduced ejection fraction for research and therapeutic purposes, which opens new spaces in the treatment of the mentioned condition.

Keywords: Acute decompensated heart failure (ADHF), Glutathione S-transferase Mu 1 (GSTM1), Heart failure with reduced ejection fraction (HFrEF) and Null gene

INTRODUCTION

Acute decompensated heart failure is a medical emergency when the heart suddenly becomes unable to pump blood effectively. Numerous things, such as excessive blood pressure, heart attacks, infections, or damage to the heart muscle, can result in this illness. Acute decompensated heart failure (ADHF) is a dangerous ailment that could be life-threatening and needs immediate medical care. ADHF is a common cause of hospitalization, particularly among older adults. Approximately 1 million hospitalization in the United States are estimated to be due to ADHF each year. The incidence of ADHF is increasing, likely due to population aging and rising rates of risk factors such as diabetes, high blood pressure, and obesity (Yancy *et al*., 2017). ADHF can occur in individuals with either preserved ejection fraction (HFpEF) or reduced ejection fraction (HFrEF). However, ADHF is more commonly associated with HFrEF. Reduced ejection fraction is when the heart muscle is weakened and cannot move blood out effectively, leading to a decreased ejection fraction. HFrEF is a common reason for ADHF, particularly where patients have a history of HF. While ADHF can occur in patients with HFpEF, it is less prevalent than in individuals with HFrEF (Yancy *et al*., 2017; Rosano *et al*., 2022). *GSTM1* (Glutathione S-Transferase Mu 1) plays a crucial role as an enzyme in the metabolism of xenobiotics and carcinogens in the body. It belongs to the glutathione S-transferase family of enzymes, which are active in detoxifying a variety of endogenous and exogenous substances by catalyzing the conjugation of glutathione to electrophilic substrates. The *GSTM1* gene encodes for the *GSTM1* enzyme and is located on

chromosome 1p13.3. The *GSTM1* enzyme is highly expressed in the liver, which is the body's main site of xenobiotic metabolism. Individuals can have different variations in the *GSTM1* gene, resulting in varying levels of enzyme activity (Tang *et al*., 2014). Two different supergene families encode the cytosolic and membrane-bound versions of glutathione S-transferase, respectively. Currently, alpha, kappa, mu, omega, pi, sigma, theta, and zeta are the eight different classes of soluble cytoplasmic mammalian glutathione S-transferases that have been found. A cytoplasmic glutathione S-transferase from the mu class is produced by this gene (Navarro *et al*., 2009). It is also present in other tissues, such as the lung, kidney, and small intestines. In addition to its detoxification function, *GSTM1* has been shown to have other roles in the cell, including modulation of cell signaling pathways and inhibiting apoptosis (Hayes *et al*., 2005). The glutathione-S-transferases play a role in conjugating prooxidant species with glutathione to facilitate the elimination of reactive oxygen species. *GSTM1* is the gene encoding one such isoenzyme. This gene copy number has undergone gene deletion and expansion, so chromosomes have no copies, 1 copy or, in rare cases, 2 copies of the gene. Two copies of the active allele are required for enzymatic activity (haploinsufficiency); those homozygous for the null allele, *GSTM1*(0), completely lack enzyme production. Individuals with the inactive *GSTM1* genotypes (*GSTM1* 0/0 or 1/0) have been found to be at higher risk of common malignancies, atherosclerosis, coronary heart disease, and CKD progression (Hung *et al*., 2022). The present study aimed to ascertain the connection between polymorphisms in the *GSTM1* gene and HFrEF patients.

MATERIALS AND METHODS

Samples collected

This study included sixty (60) ADHF patients aged 25 to 94. All patients' names, ages, genders, occupations, addresses, and medical histories were gathered. These patients were chosen from the Ramadi Teaching Hospital and the Ibn Al-Baitar Specialized Center for Cardiac Surgery. Each case was chosen after a cardiologist performed a clinical evaluation. For the collection of blood samples, thirty (30) volunteers who appeared to be in good health but had no prior history of ADHF were chosen as the control group. The patient groups were matched based on age, sex, place of residence, and environment. Participants in the study gave their signed, informed consent.

Blood specimen

Blood samples were taken from the patient and control groups; two milliliters of blood were taken by venipuncture, sterilized with an antiseptic solution, deposited in an EDTA tube, and stored at -20°C for molecular research (Clark *et al*., 2003).

Molecular analysis DNA extraction

After being thawed, the blood samples were allowed to cool to room temperature, and then, with the help of Easy Pure Blood Genomic DNA, the DNA was isolated from whole blood.

Agarose gel electrophoresis of DNA

After the extraction of DNA, the DNA was determined using pre-PCR electrophoresis.

Methods of PCR for detection of specific genes Primers Solutions

GSTM1 is listed in Table 1. The developed primers were offered by the BIONEER Company as lyophilized products in a range of picomol concentrations. They were previously studied and are based on the National Center for Biotechnology Information (NCBI).

Preparation PCR mixture

Table 2 shows the volume of the PCR mixture used in the study. Twenty-five µl of the PCR reaction were made up of 2x Easy Taq PCR master mix (Trans Gene Biotech), primer solution, deionized water, and template DNA.

The reaction mix was prepared for assay prior to transfer to the optical reaction plate for heat cycling. The tube contents were swiftly centrifuged after being sealed to spin them down and eliminate air bubbles from the solution.

Table 1. Sequences of PCR primers and molecular sizes of PCR products

Gene		Primers sequences	TM (C^0)	Product (bp)
GSTM1	F	GAACTCCCTG AAAAGCTAAA GC	56.6	215
	R	GTT-GGGCTCAAAT ATACGGTGG	58.4	215

Table 2. Volume of each component used in Multiplex –PCR

Component	Volume of reaction mixture for a single tube
Master Mix	12.5
DNA template µl	3.5 µl
Forward primer(10 Picomol) of GSTM1	1 µl
Reverse primer(10 Picomol) of GSTM1	1 µl
Nuclease free water	7 µl
Total volume	25 µl

Table 3 shows a list of the thermal cycling conditions that were used.

Table 3. Thermal cycling conditions

Step	Temperature (C^0)	Time	No. of cycle
Initial denaturation	95	5 min	1
Denaturation	94	2 min	35 Cycle
Annealing	59	1 min	
Extension	72	1min	
Final extension	72	10 min	1
Hold Temperature	4	4 min	-

While the remaining product was kept at -20°C, five microliters of it were electrophoresed.

Ethics statement

This study was approved (approval number 47, May 7, 2023) by the Medical Ethics Committee of the University of Al-Anbar Governorate in Ramadi, Iraq, following the Helsinki Declaration. All research participants, including patients and their parents, provided signed informed consent.

Statistical analysis

The statistical software SPSS-22 (Statistical Package for the Social Science) was used to analyze the data. The data were represented using basic frequency and percentage measures. One Wayay Analysis of Variance (ANOVA) and the Chi-square test (X2) were used to assess the significance of variations in various percentages (quality data). Statistical significance was considered each time the P-value for the relevance check was either equal to or less than the P-value for the relevance check (0.05).

RESULTS AND DISCUSSION

The present study involved sixty (60) ADHF patients with low ejection fraction. There were 41 (68.3%) men and 19 (31.6%) women. Thirty (30) healthy participants served as the study's control group.

Based on the patients' ages, which ranged from 25 to 94, the ADHF patients were classified into three age groups. The findings show that men were more common in all age groups. A recent study found that, compared to all other age groups, the age range (46–65) had the highest frequency, as indicated in Table 4.

Table 4. Distribution of ADHF patients according to the ages

Age groups	Total No.	Male	Female	%
25 – 45	14	10	4	23.3
46 – 65	27	20	7	45
> 65	19	11	8	31.6
Total	60			100

Table 5. Distribution of GSTM1 gene polymorphism in patients and controls

GSTM1	Patient No.%	Control No.%	Chi-Square	P-value
Genotype	23(38.33)	22(73.33)		
Null Genotype	37(61.66)	8 (26.66%)	1.2056	0.27221

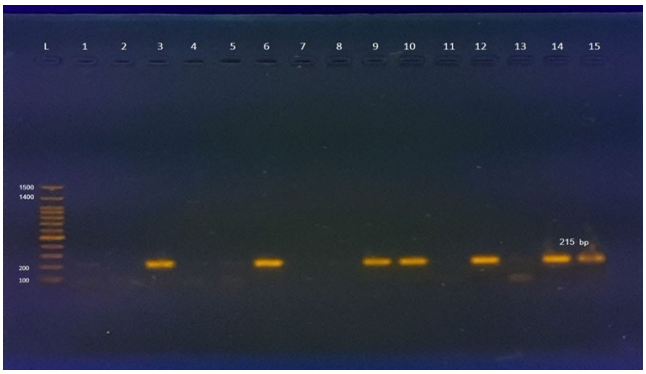

Fig.1. *Polymorphism of GSTM1 (215 bp) gene in patients produced by multiplex PCR. which analyzed on 1 % agarose gel. L : DNA Ladder (1500 bp) , Lane (3,6,9-10,12, 14-15) GSTM1 gene . Lane (1-2,4-5,7-8,11,13) GSTM1 null gene*

The present study showed that the frequency of ADHF among males 41(68.3%) was more than the females 19 (31.6 %). The results of the present study agree with Regitz-Zagrosek (2020).The other studies showed the opposite and demonstrated a higher prevalence of HFrEF in males (Fluschnik *et al*., 2021). However, other studies showed a higher prevalence in females (Swaraj *et al*., 2021). The result of a recent study may be due to the fact that ischemia is the most common cause in men, and men under 60 experience acute coronary syndromes (ACS) 3–4 times more frequently than women (Palau, Bertomeu-González *et al*., 2020). Dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM) are more frequent causes of heart failure (HF) in men than in women. Sudden cardiac death is a frequent event in HF, more common in men than in women. However, male sex remained an important predictor of HFrEF, with a hazard ratio (HR) of 2 (Ho, *et al*., 2016).

According to the age groups of patients, recent study agrees with the previous studies (Mehta and Cowie,

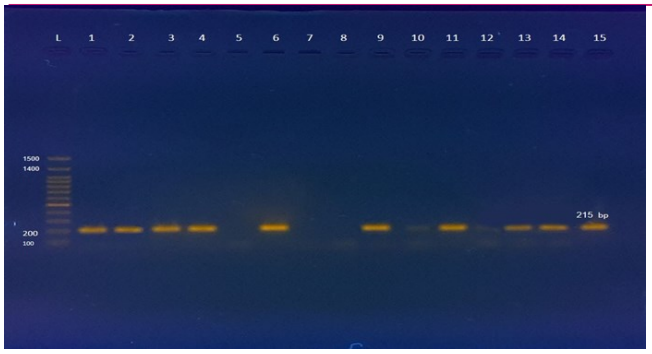

Fig. 2. *Polymorphism of GSTM1 (215 bp) gene in controls produced by multiplex PCR. which analyzed on 1 % agarose gel, L: DNA Ladder (1500 bp), Lane (1-4,6,9,11, 13-15) GSTM1 gene ;Lane (5,7-8,10,12) GSTM1 null gene.*

2006; Li *et al*. 2020). This result, which is related to age, may be because people with old age have the risk of getting a heart attack normally. This is brought on by various physical modifications to the circulatory system and the heart in general. One of these modifications is the accumulation of fatty deposits, which can occur on the artery walls. Another is the hardening of the arteries, thickened heart walls, weak heart valves and increased sodium sensitivity**.** Men are more likely than women to develop macrovascular coronary artery disease and myocardial infarction, which are well-known predisposing factors for HFrEF. This is thought to explain why men have a higher risk of developing the condition (Lam *et al.,* 2019).

Involved in redox homeostasis and glutathione conjugation-mediated ROS detoxification, GSTs are crucial phase II antioxidant genes that may contribute to oxidative stress-related illnesses (Tew and Townsend 2012). High levels of polymorphism exist in the GST genes, with GSTM1 receiving the most attention. On chromosome 1p13.3, there is GSTM1 (GST). The null polymorphism, which arises in GSTM1due to the complete gene deletion and lack of GST function, has been connected to the development and onset of various illnesses such as psoriasis, atherosclerotic cardiovascular disease (ASCVD), malignancy and type 2 diabetes mellitus (DM2) (Lee *et al*., 2022; Sobha and Ebenezar 2022). The null genotype polymorphism causes oxidative stress due to the decreased antioxidant capacity, which results in inflammation and other cellular dysfunctions in chronic disorders (Lee *et al*., 2022). The genotype GSTM1 null is linked to a higher risk of cardiovascular disease (Bhatti *et al*., 2018). Environmental (such as nutrition and exposure to pollutants) and genetic factors may also contribute to interindividual variation in the activity of GST (Binkov *et al*., 2007).

Recent epidemiological research suggested that having the GSTM1 null genotype was associated with a higher risk of developing oxidative stress-related illnesses, such as cancer, cardiovascular and respiratory diseases (Masetti *et al*. 2003). However, despite nonsignificant differences according to statistical analysis, the number of patients with the null genotype was 37 (61.66%) more than those with genotype 23(38.33%). This logical result is conducted by (Manfredi *et al*. 2007; Wang *et al*. 2019). The other study showed the opposite of it (Sobha and Kesavarao, 2023).

Conclusion

The present study showed that patients with HFrEF have a high ratio in the presence of GSTM1 null gene healthy persons. Therefore, the present study suggests that the presence of antioxidant null gene (GSTM1) has potential etiology for HFrEF, which suggests a new therapeutic strategy for preventing this condition by using the antioxidant supplement.

Conflict of interest

The authors declare that they have no conflict of interest.

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