Association of COVID-19 with Gene Expression of glutathione-S-transferase (GSTT1 and GSTM1) gene polymorphisms in Hillah Patients

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Abstract
Global health concerns have arisen due to SARS-CoV2 infection, which causes a wide spectrum of respiratory ailments, from mild to lethal, including COVID-2019 (SARS-CoV2 Infection-Induced Coronavirus Disease). A SARSCoV2 infection is associated with oxidative stress, which causes cytokine production and inflammation, as well as other pathological processes. In the lungs, glutathione S-transferase (GST) is a critical antioxidant defense enzyme that catalyzes the combination of glutathione (GSH) with electrophiles to shield cells from oxidative damage. That is why we conducted this study to see if there was a connection between the GSTM1 and the GSTT1 gene polymorphism and COVID19 susceptibility. 63 people with COVID-19 (both males and females, with an average age of 46.3 years) were enrolled from September 2021 to March 2022 from four different educational hospitals in Babylon, Al-Hillah, Al-Sadeq and Merjan. Participants had to meet the following requirements in order to be considered for the study: Positivity was determined by a reverse transcription (rt-PCR) assay for SARS-CoV-2 utilizing nasopharyngeal and oropharyngeal swabs collected in accordance with World Health Organization standards and accessible RT-PCR methods. The control group was comprised of 60 people (19 men,44 women; average age 46.3 years) who had been proven to be free of SARS-CoV-2 antibodies (IgM and IgG). It was important to select controls that had the same exposure to infection risk as the patient group in order to ensure that the groups were homogeneous in composition. As conclusion, A statistically significant relationship between GSTT1 and GSTM1 polymorphisms and an increased risk of developing COVID-19 in conjunction with lung cancer has been discovered, according to the findings. These findings suggest that environmental and genetic variables interact in a synergistic manner during the formation of lung cancer tumors.
Keywords: Covid-19; GSTM1; GSTT1; Polymorphisms; Glutathione-S-transferase; Cancer

1. Introduction

Oxidative stress may have a significant role in SARS-CoV-2 infection, according to recent research. A number of mechanisms in COVID-19, including viral receptor binding and replication, increased cytokine production, inflammation and cell signaling, are thought to be affected by oxidative stress (1 Fernandes et al., 2020). Excessive production of reactive oxygen species (ROS) in immune cells is triggered by transcription factors and pro-inflammatory genes that are activated by ROS (2 Chatterjee, 2016). [1]

This family of enzymes, glutathione S-transferases (GSTs), protects cells against xenobiotics that might be harmful to the body, as well as from reactive oxidative chemicals (3 Hayes and Strange, 2000).[3]

A mystery pneumonia was detected in Wuhan, China, in late December of this year. The coronavirus disease of 2019 has been renamed (COVID-19). More than 61 million confirmed cases and over 1.4 million deaths have been reported from around the world as a result of the fast spread of COVID-19 from China.1 Infectious disease genesis and prognosis have been linked to a variety of genetic and environmental factors, according to numerous studies (4 Williams-Blangero, et al. 2011). Finding out what causes COVID-19's rapid proliferation is really essential. Population-level awareness of relationships between population genetic backgrounds, environmental conditions, and COVID-19 epidemiologic indicators is critical for making optimal decisions about pandemic control and prevention. This should not be overlooked. [4]

Oxidative stress products are cleared from the body in different ways depending on the individual, and the GST enzyme plays a role in this process (5 Dasari, et al. 2018). Cellular GST enzymes have been classified into eight different classes in mammals, including alpha ()-GSTA; mu ()-GSTM; pi (>GSTP); omega ()-GSTO; theta (>GSTT); sigma (>GSTS); kappa (o)-GSTK; and zeta (o)-GSTZ (6 Sheehan, et al. 2001). Genome 1p13.3 and 22q11.23 are home to the most common variants of the GST genes, which are referred to as GSTM1 and GSTT1 (7 Okcu, et al. 2004). Oxidative stress-related multifactorial disorders, including as cardiovascular and respiratory diseases, can be exacerbated by the absence of enzyme activity in homozygous deletion of the GSTM1 (GSTM1/) and

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GSTT1 (GSTT1/) genes (8 Allocati, et al. 2018). As a result, in this study, we examined the relationship between GSTM1 and/or GSTT1 polymorphisms and susceptibility to COVID-19 in the North Indian population. [23]

As a result of interactions between hereditary and environmental factors, the development of oral carcinomas is considered a multifactorial illness. Alcohol and cigarette use are the most significant risk factors for this condition (IARC, 2007; Galbiatti et al., 2013; INCA, 2017). Tobacco contains chemicals and components that may cause cancer and have genotoxic effects, which may alter a person's genetic information (9 Lin et al., 2013).

The glutathione S-transferase (GST) genes GSTT1 and GSTM1, which encode enzymes involved in the biotransformation of carcinogens, also suffer from null polymorphisms caused by gene deletions. As a result, GSTT1 and GSTM1 null polymorphisms are linked to the development of oral carcinomas, as the absence of the enzymes that detoxify the metabolites of tobacco smoke, a major risk factor for this type of cancer, leads to a higher risk of developing oral and several other types of cancer (11 Ruwali et al., 2011). In research investigating the link between oral cancer and GSTT1 and GSTM1 null genotypes, results have been inconsistent, hence this meta-analysis was necessary.

2. Materials and Methods

Experimentation and sample collecting

For the research, 63 people with COVID-19 (both males and females, with an average age of 46.3 years) were enrolled from September 2021 to March 2022 from four different educational hospitals in Babylon, Al-Hillah, Al-Sadeq and Merjan. Participants had to meet the following requirements in order to be considered for the study: Positivity was determined by a reverse transcription (rt-PCR) assay for SARS-CoV-2 utilizing nasopharyngeal and oropharyngeal swabs collected in accordance with World Health Organization standards and accessible RT-PCR methods. The control group was comprised of 60 people (19 men,44 women; average age 46.3 years) who had been proven to be free of SARS-CoV-2 antibodies (IgM and IgG). It was important to select controls that had the same exposure to infection risk as the patient group in order to ensure that the groups were homogeneous in composition. Babylon University's Ethics Committee accepted this work. EDTA tubes containing 2 ml of blood from each patient were collected and kept at 20°C until they were ready to be used again.

DNA Isolation and GST Genotyping

GENEzolTM TriRNA Pure Kit was used to purify total RNA from study participants' EDTA-anticoagulated peripheral blood (Thermo Fisher Scientific, United States). Reverse transcriptase enzyme (Reverse Transcription Kit with dsDNase) was used to transform the RNA product into DNA at a temperature of 20°C (BioSharp, China).

On ice in an RNase-free tube, prepare the following reaction system

Total RNA/mRNA 0.1-2 ng, 5 times RT Master Mix RT Master Mix A random or particular primer and 4 l, 20 Oligo dT 20 l of RNase-free H2O and 1 l of RNase-free H2O This was then put into the PCR machine and run according to this program after being gently mixed with a pipette. A spectrophotometer, a gel electrophoresis check on a 1 percent agarose gel, and a Nanodrop were used to measure DNA quality and quantity at 37 C (1530 minutes) and 85 C (5min) (Thermo Fisher Scientific).

By employing real-time polymerase chain reaction (qPCR), the GSTM1 and GSTT1 null genotypes were identified. This was done by using particular primers: F5'GAACTCCCTGAAAAGCTAAAGC and R5'GTTGGGCTCAAATATACGGTGG. Reaction mixtures containing 150–200ng of genomic DNA, 5 pmol of each primer and 2x master mix (Takara) per tube were used to do PCR. The gradient Rotor-Gene Q PCR machine was used to complete the amplification (QIAGEN, Germany). To determine the null genotypes of both genes (GSTM1 and GSTT1), 2.5 percent agarose gels (Quantum Vilber, France) were used to observe the PCR results.

3. Results and Discussion

There were 46.3 years between the mean ages of the patients. This study included a total of 63 COVID-19 participants who were all enrolled in the study. Sixty-seven percent of patients had minor symptoms, while the remaining patients were suffering from serious symptoms. Only one patient died out of the entire group of severe patients, and that patient was the only one who died out of the entire group of severe patients.

Table (1) The correlation between GSTM1, GSTT1 genes polymorphism associated with Covid-19 patients and Control

Gene	Patients (63)				Total		Healthy (50)				Total	
	Male		Female				Male		Female			
	No	%	No	%	No	%	No	%	No	%	No	%
GSTM1	16	6.30	38	14.96	54	21.26	8	7.27	11	10.00	19	17.27
GSTT1	16	6.30	41	16.14	57	22.44	10	9.09	13	11.82	23	20.91
Total	32	12.60	79	31.10	111	43.70	18	16.36	24	21.82	42	38.18

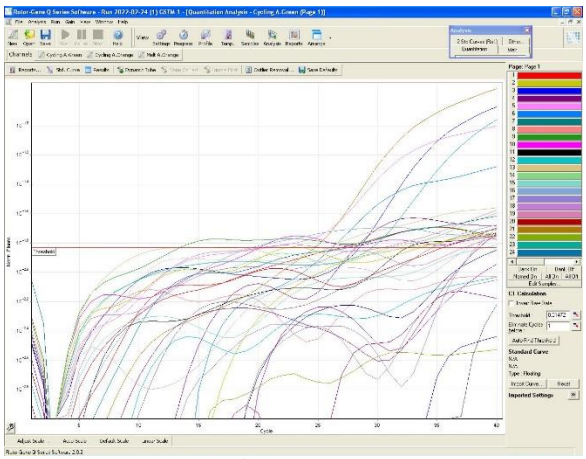

Figure 1 Rt-qPCR of GSTM1 gene using QIAGEN "RoterGen" Machine

Numerous xenobiotics and/or their Phase I metabolites are detoxified by GST-mediated GSH conjugations (12 Li et al., 2005). Oxidative stress-related multifactorial illnesses, including COVID19, are more likely to occur in people who have a null genotype for GSTM1 and GSTT1. Polymorphisms in the GST gene is associated with an increased risk of oxidative stress, which may play a significant role in the susceptibility to SARS-CoV infection and its outcome (13 Saadat et al., 2020). The formation of reactive oxygen species (ROS) caused by SARS-CoV2 disrupts the antioxidant defense system, resulting in an inflammatory milieu and significant tissue damage, both of which contribute to the death of COVID19 patients.

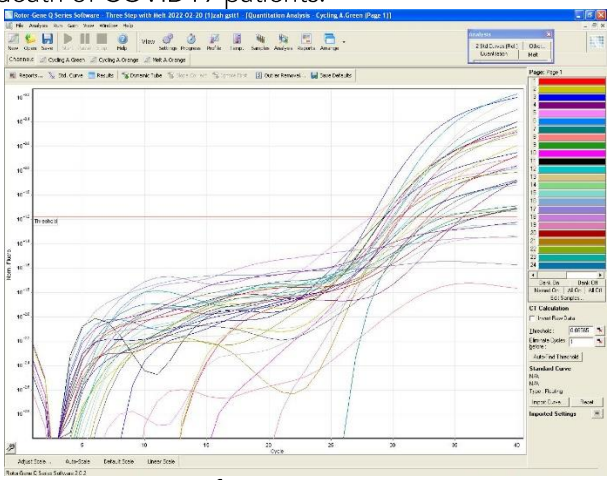

Figure 1 Rt-qPCR of GSTT1 gene using QIAGEN "Roter Gen" Machine

19 patients died (14 Huang et al., 2019). A virus-induced OS and its following effects on cells, tissues, and the organism are still a mystery.

It's true that antioxidants and ROS have a conflicting function in viral propagation (15 Khomich, et al., 2018). The GST enzyme's activity was dramatically increased in melatonin-treated rats, suggesting that it may minimize OS associated with COVID19 infection (16 Zhang et al., 2020). COVID19 patients with GSTT1/ had a greater mortality rate and a shorter overall survival rate, according to the results of this study. Oxidative stress is more widespread in patients with low or no GST activity, according to these data. People who have the GSTT1/ genotype had a higher risk of COVID19 infection than those who have the GSTT1+/+ genotype, according to Saadat25. However, the population with a low frequency of GSTT1/ genotype showed the highest number of COVID19 cases and deaths in East Asian countries. GSTT1/ alone or in combination with GSTM1/ genotype resulted in a greater drop in FEV1 (forced expiratory volume in the first second) among males independent of smoking status, according to another study. Ding and colleagues (17 Ding et al., 2019).

4. Acknowledgment

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5. Conflict of Interests

There are no competing interests declared by the authors.

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