UNIVERSIDADE ESTADUAL DO OESTE DO PARANÁ – *CAMPUS* DE FRANCISCO BELTRÃO, CENTRO DE CIÊNCIAS DA SAÚDE, PROGRAMA DE PÓS-GRADUAÇÃO *STRICTO SENSU* EM CIÊNCIAS APLICADAS À SAÚDE – NÍVEL MESTRADO

PRISCILA DA SILVA ANTUNES VIEIRA

ESTUDO DOS POLIMORFISMOS *GSTP1* (rs1695) e *TNF A-308* (rs1800629) EM PACIENTES COM COVID-19 HOSPITALIZADOS E ASSOCIAÇÃO COM DADOS CLÍNICOS

FRANCISCO BELTRÃO – PR
(MAIO/2024)

PRISCILA DA SILVA ANTUNES VIEIRA

ESTUDO DOS POLIMORFISMOS *GSTP1 (rs1695)* e *TNF A-308* (rs1800629) EM PACIENTES COM COVID-19 HOSPITALIZADOS E ASSOCIAÇÃO COM DADOS CLÍNICOS

DISSERTAÇÃO apresentada ao Programa de Pós-graduação *Stricto Sensu* em Ciências Aplicadas à Saúde, nível Mestrado, do Centro de Ciências da Saúde, da Universidade Estadual do Oeste do Paraná, como requisito parcial para obtenção do título de Mestre em Ciências Aplicadas à Saúde.

Área de concentração: Ciências da Saúde.

Orientador(a): Dra. Léia Carolina Lucio

Co-orientador(a): Dr. Fernando Rodrigo Treco

FRANCISCO BELTRÃO – PR
(MAIO/2024)

Silva Antunes Vieira, Priscila da
Estudo dos polimorfismos GSTP1 (rs1695) e TNF A-308 (rs1800629) em pacientes com COVID-19 hospitalizados e associações com dados clínicos / Priscila da Silva Antunes Vieira; orientadora Léia Carolina Lucio; coorientadora Fernando Rodrigo Treco. -- Francisco Beltrão, 2024.
55 p.

Dissertação (Mestrado Profissional Campus de Francisco Beltrão) -- Universidade Estadual do Oeste do Paraná, Centro de Ciências da Saúde, Programa de Pós-Graduação em Ciências Aplicadas à Saúde, 2024.

1. SARS-CoV-2. 2. Polimorfismo de nucleotídeo único. 3. Glutationa S-transferase. 4. Citocina. I. Lucio, Léia Carolina, orient. II. Treco, Fernando Rodrigo , coorient. III. Título.

FOLHA DE APROVAÇÃO

PRISCILA DA SILVA ANTUNES VIEIRA

ESTUDO DOS POLIMORFISMOS *GSTP1 (rs1695)* e *TNF A-308* (rs1800629) EM PACIENTES COM COVID-19 HOSPITALIZADOS E ASSOCIAÇÃO COM DADOS CLÍNICOS

Essa dissertação foi julgada adequada para obtenção do título de Mestre em Ciências Aplicadas à Saúde e aprovada em sua forma final pelo(a) Orientador(a) e pela Banca Examinadora.

BANCA EXAMINADORA

Orientador (a): Prof (a). Dr (a) Léia Carolina Lúcio.
UNIOESTE

Membro da banca: Prof (a). Dr (a) Claudicéia Risso Pascotto.
UNIOESTE

Membro da banca: Prof (a). Dr (a) Nédia de Castilhos Ghisi.
UTFPR

FRANCISCO BELTRÃO, PR
Maio/2024

AGRADECIMENTOS

Inicio agradecendo à Prof. Dra. Léia Carolina Lucio, grande orientadora desta dissertação, por toda dedicação, paciência, sabedoria, compreensão e, acima de tudo, exigência. E com certeza, exemplo de profissional e pessoa, que levarei sempre comigo com grande carinho e respeito. Também ao Co-orientador Dr. Fernando Rodrigo Treco, ao membro interno da banca Dra. Claudicéia Risso Pascotto, e ao membro da banca externo ao PPGCAS, Dra. Nédia de Castilhos Ghisi.

Agradeço à minha colega e parceira mestranda Marcieli Borba do Nascimento, que conduziu com êxito as atividades do laboratório, possibilitando o desenvolvimento da pesquisa, além de colaborar imensamente no restante do trabalho.

Em especial agradeço minha família, meu esposo Marcelo e minha filha Milena , que puderam compartilhar comigo uma rotina acelerada e compreender minha ausência.

DEDICATÓRIA

Dedico este trabalho à minha amada mãe Regina (in memorian) que sempre me incentivou a estudar e esteve ao meu lado até fevereiro de 2021.

LISTA DE ILUSTRAÇÕES

LISTA DE TABELAS

LISTA DE ABREVIATURAS E SIGLAS

A - Adenina
ACE2- enzima conversora da angiotensina 2
CEP- Pesquisa envolvendo Seres Humanos
CONEP-Comitê de Ética em Pesquisa
COVID-19 - Doença do Coronavírus 2019
DNA – Ácido Desoxirribonucleico
DPOC- Doença Pulmonar Obstrutiva Crônica
EDTA - Ácido Etilenodiamino Tetra-Acético
EHW - Equilíbrio de Hardy-Weinberg (EHW)
FIN- ficha Individual de Notificação
G- Guanina
GAL- Gerenciador de Ambiente Laboratorial
GST- Glutationa
GSTP1- glutationa S-transferase P1
GSTs- Glutationa S-transferase
GSUS- Sistema de Gestão Hospitalar e Ambulatorial
HAS- Hipertensão Arterial Sistêmica
HRS-WAP - Hospital Regional do Sudoeste Dr. Walter Alberto Pécoits
Ile- Isoleucina
Lacen- Laboratório Central do Estado
PCR – Reação da Polimerase em Cadeia
OMS - Organização Mundial de Saúde
qRT-PCR – Quantitativa Transcriptase Reversa - Reação da Polimerase em Cadeia
S- Glicoproteína Spike
SARS-Cov- Síndrome Respiratória Aguda Grave causada pelo Coronavírus
SARS-Cov-2- Síndrome Respiratória Aguda Grave causada pelo Coronavírus 2
SNP- Polimorfismo de Nucleotídeo Único
SpO2 – Saturação de oxigênio
SPSS - Statistical Package for the Social Sciences
SDR - Síndrome do Desconforto Respiratório
SRAG - Síndrome Respiratória Aguda Grave
SRC - cytokine release syndrome (síndrome de liberação de citocinas)

TNF A -308 - Fator de necrose tumoral A- 308

TNF-fator de necrose tumoral

UTI- Unidade de Terapia Intensiva

Val-Valina

ESTUDO DOS POLIMORFISMOS *GSTP1* (rs1695) e *TNF A-308* (rs1800629) EM PACIENTES COM COVID-19 HOSPITALIZADOS E ASSOCIAÇÃO COM DADOS CLÍNICOS

Resumo

O SARS-CoV-2, coronavírus agente da COVID-19, rapidamente disseminou-se em todo o mundo, progredindo com manifestações clínicas diversas, desde a forma assintomática, leve, moderada à grave, com grande desfecho de óbito. Ainda é um desafio a compreensão da fisiopatologia do SARS-CoV-2 para as medidas de controle e manejo clínico da doença. Dentre os fatores que interferem na evolução da COVID-19, estão idade, sexo, hábitos de vida e presença de comorbidades, além de fatores genéticos que são alvo de investigações e podem interferir na susceptibilidade e evolução da doença. O principal objetivo do estudo foi determinar a frequência alélica e genotípica de *GSTP1* (rs1695) e *TNF A-308* (rs1800629) nos pacientes hospitalizados com COVID-19 e verificar associação dos polimorfismos com a gravidade, comorbidade e o desfecho da hospitalização dos pacientes do sul do Brasil. A amostra foi composta por 236 pacientes positivos para COVID-19 internados em um hospital de referência do sudoeste paranaense. Foi coletado 5 mL de sangue dos indivíduos para caracterização genética, utilizando as técnicas T-ARMS-PCR (tetra amplification refractory mutation system by Polymerase Chain Reaction) para *rs1695 e* ARMS-PCR (amplification refractory mutation system by PCR) para rs1800629. Para coleta de dados sociodemográficos, clínicos e das comorbidades foi realizada busca em prontuários médicos dos pacientes. Posteriomente, foram conduzidas análises bivariada e multivariada, incluindo a regressão logística. Os resultados mostraram que 58,1% da amostra eram homens e 41,9% mulheres; 63% eram casos graves e 37% moderados; o desfecho de alta hospitalar foi de 64% e 36% foram a óbito. Em relação ao polimosrfismo genético, o alelo ancestral foi o mais frequente em rs1695 (A = 71%) e rs1800629 (G = 61%) e, que os genótipos AA (rs1695) e AG (rs1800629) foram, respectivamente, 48,73% e 67,81%. Para rs1695 tanto o alelo A (OR = 2,9; IC95%= 1,093–7,884; p=0,033) quanto o genótipo AG (OR = 3,3; IC95%= 1,185–9,375; p= 0,022), ampliam as chances de gravidade para COVID-19. Para *o* polimorfismo rs1800629 nenhuma associação significativa foi observada. Com relação ao desfecho hospitalar, comorbidades, saturação de oxigênio e tempo de hospitalização nenhuma associação foi observada com os

polimorfismos investigados. Logo, o estudo conclui que tanto o alelo A quanto o genótipo AG de rs1965 de *GSTP1* são marcadores sugestivos de gravidade da COVID-19 na população sul do Brasil. E nenhuma relação pode ser estabelecida para o polimorfismo rs1800629 de *TNF A-308.*

Palavras-chave: SARS-CoV-2, SNPs, Glutationa-S-Transferase, citocina, comorbidades.

STUDY OF *GSTP1* (rs1695) and *TNF A-308* (rs1800629) POLYMORPHISMS IN HOSPITALIZED COVID-19 PATIENTS AND ASSOCIATION WITH CLINICAL STATUS

Abstract

SARS-CoV-2, the new coronavirus agent of COVID-19, quickly spread throughout the world, progressing with diverse clinical manifestations, from asymptomatic, mild, moderate to severe forms, with a major outcome of death. It is still a challenge to understand the pathophysiology of SARS-CoV-2 for control measures and clinical management of the disease. Among the factors that affect the evolution of COVID-19 are: comorbidities, age, sex and lifestyle habits. Genetic factors are also the subject of investigations and may interfere with the susceptibility and evolution of the disease. The main objective of the study was to determine the allelic and genotypic frequency of *GSTP1* (rs1695) and *TNF A-308* (rs1800629) in patients hospitalized with COVID-19 and verify the association of polymorphisms with the severity, comorbidity, and outcome of hospitalization of patients in southern Brazil. In total, 236 patients positive for COVID-19 admitted to a reference hospital in southwestern Paraná participated in the research. A blood sample was collected from the individuals for genetic characterization using the techniques T-ARMS-PCR (tetra amplification refractory mutation system by Polymerase Chain Reaction) for rs1695 and ARMS-PCR (amplification refractory mutation system by PCR) for rs1800629. At the same time, a search was carried out in the patients' medical records to collect sociodemographic, clinical and comorbidity data. Subsequently, bivariate and multivariate analyses were conducted, including logistic regression. were Of the population, 58.1% were men and 41.9% were women; 63% were severe cases, and 37% were moderate; the hospital discharge outcome was 64%, and 36% died. The results showed that the ancestral allele was the most frequent in rs1695 (A = 71%) and rs1800629 (G = 61%) and that the genotypes AA (rs1695) and AG (rs1800629) were, respectively, 48.73% and 67.81%. For rs1695, both the A allele (OR = 2.9; 95%CI= 1.093–7.884; p=0.033) and the AG genotype (OR = 3.3; 95%CI= 1.185–9.375; p= 0.022) increase the chances of severity for COVID-19. For the rs1800629 polymorphism, no significant association was observed. Regarding hospital outcome, comorbidities, oxygen saturation and length of hospitalization, no association was observed with the investigated

polymorphisms. Therefore, the study concludes that both the A allele and the AG genotype of *GSTP1* rs1965 are suggestive markers of COVID-19 severity in the southern Brazilian population. And no relationship can be established for the rs1800629 polymorphism of TNF A-308.

keywords: SARS-CoV-2; Single-nucleotide polymorphism; Glutathione S-transferase; Cytokine; Comorbidity.

SUMÁRIO

.

1. INTRODUÇÃO

A pandemia de COVID-19 (Doença do coronavírus) teve duração de pouco mais de três anos, com o fim da Emergência de Saúde Pública de Importância Internacional declarada em maio de 2023 (Kumar; Srivastava; Nand, 2023). O SARS-CoV-2 (Síndrome respiratória aguda grave causada pelo novo coronavírus), foi descoberto em dezembro de 2019 na província de Wuhan na China, é o sétimo coronavírus a infectar o homem provocando uma pneumonia atípica, denominada COVID-19 (Wu *et al.*, 2020; Wang *et al.*, 2023). O panorama mundial da COVID-19 ultrapassou 770 milhões de casos confirmados com taxa de letalidade de 0,9% (Topcuoglu, 2020). No Brasil, o Ministério da Saúde divulgou mais de 37 milhões de casos positivos com taxa de letalidade de 1,8% pela doença até meados de outubro de 2023 (SESA, 2023).

O vírus SARS-CoV-2 possui características únicas que o distingue dos demais coronavírus, como a alta transmissibilidade, provocando aumento do número de pessoas infectadas em curto período de tempo e a evolução para a síndrome respiratória aguda grave (SRAG) (Ciotti *et al.*, 2020; Li *et al.*, 2020). Esse quadro progrediu, em muitos casos, para insuficiência respiratória com necessidade de cuidados intensivos, resultando no desfecho de óbito (Grasselli *et al.*, 2020). O estudo do genoma do SARS-CoV-2 revelou sua adaptação aos receptores das células humanas, propiciando uma rápida infecção (Pirola; Patanè, 2023). Ele possui uma configuração de domínio de ligação ao receptor celular humano semelhante ao do SARS-CoV, a enzima conversora da angiotensina 2 (ACE2) (Malik, 2020), uma proteína transmembrana expressa na superfície de diversas células do corpo, como o epitélio do sistema respiratório (Bernstein *et al.*, 2013). A proteína *Spike (S)* presente no capsídeo viral é uma estrutura necessária para a ligação no receptor celular ACE2 e, tem como característica flexibilidade molecular com diferentes disposições, as quais definem junto com as mutações do genoma viral, as variantes de SARS-CoV-2 (Turoňová *et al.*, 2020). Além do que, as mutações também estão associadas ao aumento da virulência, da transmissibilidade e exacerbação da doença (Janik *et al.*, 2021).

A principal via de transmissão do SARS-CoV-2 é a respiratória (Lamers; Haagmans, 2022) e, por isso, as recomendações foram o isolamento social e o uso de máscaras (Hwang *et al.*, 2020; Wei *et al.*, 2021). Assim como outras doenças

virais, a COVID-19 pode ser assintomática ou desencadear diversos sintomas (Cabrera Martimbianco *et al.*, 2021; Lima, 2020; Struyf *et al.*, 2022). Logo, o quadro clínico do indivíduo pode ser classificado em leve, moderado ou grave, dependendo do tipo de manifestação clínica (Elezkurtaj *et al.*, 2021). O índice de saturação de oxigênio (SpO_2) associado aos sintomas de alerta como febre e falta de ar foi um parâmetro muito utilizado na classificação de gravidade da doença (OMS, 2021).

Além disso, outros preditores de gravidade da COVID-19, como idade, sexo e a presença de comorbidade, podem estar relacionados à desregulação da resposta imunoinflamatória do indivíduo frente à infecção viral (Torres *et al.*, 2022; Purwanty; Permata, 2023). Outro fator que chamou a atenção foi a evolução da gravidade em indivíduos sem histórico de doença pré-existente, o que fez considerar a hipótese do perfil genético individual como fator para a severidade da COVID-19 (Orlewska *et al.*, 2023).

Os danos ocasionados pela tempestade de citocinas na COVID-19 foram demonstrados em estudos por autópsias que apontaram para relação delas com a Síndrome do Desconforto Respiratório (SDR) (Barnes *et al.*, 2020). Em pacientes graves observou-se aumento dos níveis séricos de citocinas pró-inflamatórias que pôde desencadear uma resposta inflamatória sistêmica conhecida como a síndrome de liberação de citocinas (SRC) (Guo *et al.*, 2022). O TNF A é uma citocina pró-inflamatória multifuncional que pertence à superfamília do fator de necrose tumoral (TNF) (Thomas, 2001) sendo considerada uma das principais citocinas relacionadas aos processos inflamatórios e imunes, agindo em diferentes tecidos, auxiliando na defesa do organismo contra microrganismos invasores (Vitale; Ribeiro, 2007).

Toda essa condição propiciada pela SRC provoca estresse celular e tecidual que tende a ser equilibrado também por enzimas da família das glutationa-S-transferase (GSTs). Estas enzimas estão envolvidas no controle oxidativo das células e exercem a importante função na patobiologia de diversas doenças incluindo as do aparelho respiratório (Gilliland *et al.*, 2002; Silva *et al.*, 2021a). Dentre as classes de GSTs a glutationa-S-transferase P1 (*GSTP1*) é uma das principais enzimas com esse perfil atuante no pulmão (Gilliland *et al.*, 2002; Orlewska *et al.*, 2023).

Neste contexto, os polimorfismos de nucleotídeo único (SNPs) são a forma mais comum de variação genética humana, sendo uma ferramenta para o mapeamento das características genéticas do indivíduo (Chakravorty; Hegde, 2018). A presença de SNPs em genes ou em regiões promotoras podem estar relacionadas

a progressão de determinada doença no indivíduo ou à susceptibilidade, inclusive, do agente patogênico (Schmiedel *et al.*, 2018; Logsdon; Vollger; Eichler, 2020). Tanto o *GSTP1* quanto o *TNF A* possuem regiões polimórficas, que podem interferir na resposta imunológica do organismo (Cui *et al.*, 2020; Abdulfattah; Samawi, 2023). Para o gene *GSTP1*, localizado no cromossomo 11 com aproximadamente 2,8kb e constituido por sete exons, os SNPs mais estudados são os do éxon 5 Ile105Val (rs1695) e o do éxon 6 Ala114Val (rs1138272) (Yang *et al*., 2023). O polimorfismo do éxon 5 (rs1695) é uma troca de base na posição 105 do gene, com substituição (transição) da A (adenina) pela G (guanina), trocando o aminoácido isoleucina por valina (Ile105Val) (rs1695). Com relação ao conjunto gênico *TNF A,* grande parte dos SNPs desse gene estão em sua região promotora e pressupõe-se que afetem a suscetibilidade ou gravidade de diferentes doenças (El-Tahan; Ghoneim; El-Mashad, 2016; Moura *et al*., 2019; Ahmed *et al.,* 2020; Feyda Nursal *et al.,* 2020). Especificamente, o SNP rs1800629 do TNF-A localizado na posição -308 (*upstream*) leva a substituição (transição) da guanina (G) pela adenina (A) e, na população, o alelo -308A (variante) tem sido prevalente comparado ao alelo -308G (ancestral) (Santiago *et al*., 2021; Abdulfattah; Samawi, 2023).

Na COVID-19, essas variações polimórficas no genoma do hospedeiro vêm sendo investigadas, incluindo os genes GSTs e TNF (Padula *et al.*, 2020; Abbas *et al.* 2021; Coric *et al*., 2021; Orlewska *et al.*,2023). Pacientes poloneses com genótipo AG (Ile/Val) para *GSTP1* (rs1695) apresentaram quase três vezes mais risco de severidade da doença comparado aos outros genótipos (Orlewska *et al.*, 2023). Em contrapartida, no trabalho de Coric et al. (2021) com a população sérvia o genótipo heterozigoto esteve associado a menor chance de desenvolver a COVID-19 (Coric *et al*., 2021). Ainda outro estudo, também com os sérvios, avaliou a relação entre *GSTP1* (rs1695) e o extresse oxidativo exacerbado pela doença e não encontrou resultado significativo (OR = 1,02, *p* = 0,978) (Markovic *et al.,* 2023). Considerando o polimorfismo de TNF A -308 (rs1800629) na população egípcia, indivíduos com alelo A e com genótipo AA possuem, respectivamente, risco aumentado para COVID-19 e maior severidade da doença (Saleh *et al.*, 2022). Em estudo cubano, indivíduos com o alelo A apresentaram mais sintomas da doença, porém, a gravidade foi evidenciada em pessoas com o alelo G, normalmente assintomáticos (OR = 0,96; IC 95% 0,51–1,79) (Sotomayor-Lugo *et al.*, 2022). Por outro lado, um estudo de meta-análise demonstrou ausência de relação do *TNF A-308* (rs1800629) com a gravidade da

COVD-19 (Pecoraro; Cuccorese; Trenti, 2023).

Diante do exposto, percebe-se que a presença destes polimorfismos genéticos na população podem estar relacionados aos diferentes desfechos de algumas doenças. Ademais, quando correlatos, em muitos casos, ampliam o risco e o agravo a depender da área geográfica e etnia das populações. Desta forma, conhecer a influência de alguns SNPs na COVID-19, em populações brasileiras, pode auxiliar o prognóstico e evolução clínica do paciente de forma personalizada. Assim, visto a importância destes SNPs para área da saúde, o presente estudo buscou determinar a frequência alélica e genotípica de *GSTP1* (*rs1695)* e *TNF A-308 (rs1800629)* nos pacientes hospitalizados com COVID-19 e verificar associação dos SNPs com a gravidade, presença de comorbidade e o desfecho da hospitalização dos pacientes do sul do Brasil.

2. OBJETIVOS

2.1 Geral

Determinar a frequência alélica e genotípica de *GSTP1* (rs1695) e *TNF A-308* (rs1800629) nos pacientes hospitalizados com COVID-19 e verificar associação dos SNPs com a gravidade, presença de comorbidade e o desfecho da hospitalização dos pacientes.

2.2 Específicos

- Correlacionar as frequências alélilcas e genotípicas com os casos graves e moderados de COVID-19.
- Investigar a associação dos polimorfismos rs1695 e rs1800629 com o desfecho da hospitalização pela COVID-19, alta ou óbito.
- Verificar associação das frequências alélicas e genotípicas com as comorbidades obesidade, diabetes mellitus, hipertensão arterial sistêmica, doença pulmonar obstrutiva crônica e complicações vasculares.
- Associar a presença do polimorfismo genético rs1695 e rs1800629 com o nível de saturação de oxigênio (SpO_2) e tempo de hospitalização.

3. METODOLOGIA

3.1 População e desenho do estudo

Pesquisa de caráter quantitativa, descritiva e transversal desenvolvida no município de Francisco Beltrão, sudoeste do Paraná. A população incluiu pacientes internados entre agosto de 2020 à agosto de 2021, inicialmente por suspeita e posterior confirmação de COVID-19, no Hospital Regional do Sudoeste Dr. Walter Alberto Pécoits (HRS-WAP), referência para COVID-19 na região.

Os critérios para a inclusão dos participantes no estudo foram indivíduos com resultados positivos para SARS-CoV-2, a partir do exame padrão qRT-PCR e hospitalizados, com permanência mínima de um dia. Como critério de exclusão, foram aqueles que mesmo suspeitos apresentaram resultado negativo para SARS-CoV-2 e os que não permaneceram hospitalizados. O projeto recebeu aprovação pelo Comitê de Ética em Pesquisa envolvendo Seres Humanos (CEP) com parecer nº 4.224.011 e CAAE: 31837720.5.0000.0107 (Anexo I).

3.2 Coleta de dados e de amostra sanguínea

Os dados dos 236 indivíduos, que fizeram parte da amostra, foram coletados durante a internação.Como instrumento de coleta uma uma ficha com informações dos pacientes foi preenchida, utilizando os dados obtidas através do prontuário médico, da ficha Individual de Notificação (FIN) obtida do Sistema de Informação de Agravos de Notificação, do sistema GAL/Lacen o Notifica COVID e através do programa Sistema de Gestão Hospitalar e Ambulatorial (GSUS). Dentre as informações estão idade, sexo, sintomas, comorbidades, classificação da gravidade da doença (moderado ou grave conforme definição estabelecida pela OMS), tempo total de hospitalização, tempo de assistência na Unidade de Terapia Intensiva (UTI), índice de saturação de O_2 (SpO_2), necessidade de ventilação não invasiva e invasiva, alta hospitalar ou óbito.

Para análise laboratorial foi coletado 05 ml de sangue venosode cada indivíduo, em tubo com o anticoagulante EDTA (ácido etilenodiamino tetra-acético), homogeneizado e depositado em uma caixa térmica com gelo até o armazenamento, no Laboratório de Biologia Molecular e Citogenética Humana da Universidade Estadual do Oeste do Paraná, UNIOESTE, onde foram acondicionadas em freezer -

20ºC até o processamento. Ressaltamos que, o sangue foi coletado durante o processo da hospitalização dos indivíduos, por profissionais assistenciais dos setores de referência à COVID-19 do HRS-WAP, que já realizavam diariamente coleta de material para análise laboratorial da rotina de acompanhamento desses pacientes.

3.3 Isolamento do material genético

A extração de DNA total de cada indivíduo foi realizada a partir de uma alíquota de 200µl da amostra original de sangue, seguindo o protocolo QIAamp® DNA Mini Kit, DNA Purification from Blood or Body Fluids (Spin Protocol) (Qiagen, Alemanha) conforme instruções do fabricante e armazenados em freezer a -20°C. Após a extração do DNA, foi realizada a detecção da presença de um segmento de aproximadamente 268pb do gene β-globina humano sintetizado a partir dos iniciadores GH20 (5′-GAAGAGCCAAGGACAGGTAC-3′) e PC04 (5′-CAACTTCATCCACGTTCACC-3′), para verificar a presença e qualidade do DNA ds amostras. As condições de amplificação para cada reação foi 190 nM de dNTPs, 500 nM de cada iniciador, 2 mM de MgCl2, Tampão (200 mM Tris-HCL, 500 mM KCl), 1,25 U de DNA polimerase (Ludwig™) e cerca de 50 ng de DNA submetido a sequência de etapas de ciclagem: 94ºC por 10 minutos; 37 ciclos de 94º C por 1 minuto, 55ºC por 1 minuto, 72ºC por 1 minuto; 72ºC por 10 minutos (Trugilo *et al.*, 2019).

3.4 Genotipagem *GSTP1* (rs1695) e *TNF A-308* (rs1800629)

O método utilizado para determinar o polimorfismo genético *GSTP1* (rs1695) foi o T-ARMS-PCR (tetra amplification refractory mutation system by Polymerase Chain Reaction) baseado no protocolo de Hashemi *et al. (*2012) e para determinar o polimorfismo *TNF A-308* (rs1800629) foi utilizado a ARMS-PCR (Amplification Refractory Mutation System by Polymerase Chain Reaction) (Ahmed *et al.,* 2020).

Para rs1695 foram utilizados os pares de iniciadores: *forward outer* 5'-CAGGTGTCAGGTGAGCTCTGAGCACC-3’ e *reverse outer* 5'-ATAAGGGTGCAGGTTGTGTCTTGTCCCA-3’ para o alelo A (isoleucina) e *forward inner* 5'-CGTGGACCTCCTCCGCTGCAAATCCA-3' e *reverse Inner* 5'-GCTCACATAGTTGGTGTAGATGAGGGATAC-3' ´para o alelo G (valina). As etapas de ciclagem incluíram: desnaturação inicial a 95ºC por 5 minutos, seguindo para 30 ciclos de 95ºC por 35 segundos, 62ºC por 35 segundos, 72°C por 50 segundos,

finalizando a 72ºC por 10 minutos (Hashemi *et al*.,2012). Os amplicons gerados foram de 233pb para o alelo A, 290pb para o alelo G e 467pb para primers externos/banda controle.

Para o estudo do SNP *TNF A-308* (rs1800629) foram utilizados um iniciador comum aos dois alelos o *Forward* 5'- TCTCGGTTTCTTCTCCATCG – 3'; e o *Reverse* 5' – ATAGGTTTTGAGGGGCATGA – 3' para Alelo A e, o *Reverse* 5` - ATAGGTTTTGAGGGGCATGG – 3' para Alelo G. Os alelos A e G foram avaliados separadamente pois possuem o mesmo tamanho de amplicon (184 pb) (Ahmed *et al.*, 2020) (Figura 2). A ciclagem empregada foi desnaturação inicial a 95ºC por 2 minutos, seguindo para 30 ciclos de 95ºC por 15 segundos, 65ºC por 50 segundos, 72°C por 1 minuto, finalizada a 72ºC por 5 minutos (Ahmed *et al.,* 2020).

Os genótipos de ambos os SNPs foram determinados pela migração dos produtos em gel de agarose a 2%, visualizados sob luz UV e fotodocumentados (Figura 1 e 2).

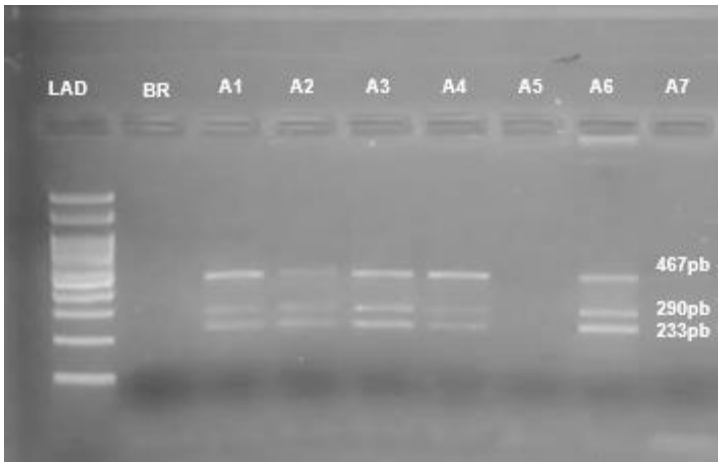


Figura 1 – Imagem do gel de agarose a 2% com representação do polimorfismo *GSTP1.* LAD (padrão de peso molecular de 100pb); BR (branco ou controle negativo); A1, A2, A3, A4, A5, A6 e A7 são as amostras. Peso molecular 467pb para o controle, 290pb para alelo G e 233pb para alelo A.

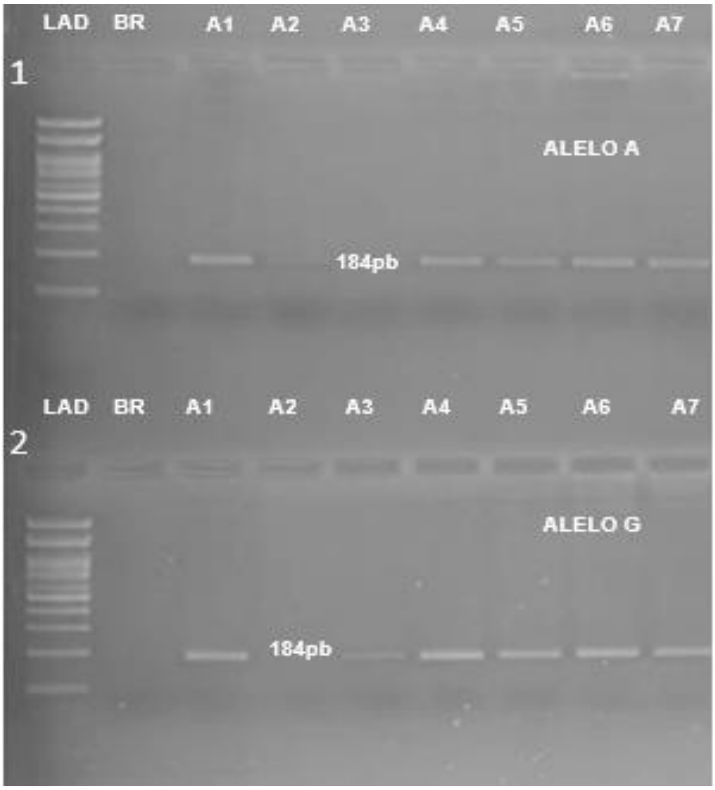


Figura 2 – Imagem do gel de agarose a 2% com representação do polimorfismo *TNF A-308* (rs1800629). LAD (padrão de peso molecular de 100pb); BR (branco ou controle negativo); A1, A2, A3, A4, A5, A6 e A7 são as amostras. Alelo A (1) e Alelo G (2), ambos com fragmento de 184pb.

3.5 Análise estatística

Os dados foram tabulados e analisados no *software Statistical Package for the Social Sciences* (SPSS) versão 24.0. As variáveis contínuas tiveram a distribuição normal verificada pelo teste de Kolmogorov-Smirnov ($p>0,05$) e, assim como, as demais foram categorizadas. Foram determinadas as frequências alélicas e genotípicas e verificado o Equilíbrio de Hardy-Weinberg (EHW) pelo teste Qui-quadrado. Este também foi utilizado, com correção de continuidade de Yates e teste exato de Fisher, para comparações entre variáveis categóricas independentes, a gravidade do caso, o desfecho de óbito e alta hospitalar e de comorbidades com os SNPs. As variáveis independentes que apresentaram significância menor que 0,20 ($p<0,20$) nas análises bivariadas seguiram para regressão logística, método *stepwise*, considerando intervalos de confiança de 95% e $p>0,05$ e determinação da razão de chance (OR). Para verificar a existência de relação entre os polimorfismos genéticos e o nível de saturação de O_2 e tempo de hospitalização foi realizado teste t de *Student*, considerando $p<0,05$.

4. REFERÊNCIAS

ABBAS, M. *et al.* Association of *GSTM1* and *GSTT1* gene polymorphisms with COVID-19 susceptibility and its outcome. **Journal of Medical Virology**, v. 93, n. 9, p. 5446–5451, set. 2021.

ABDULFATTAH, S. Y.; SAMAWI, F. T. Estimating the role of single‐nucleotide polymorphism (rs1800629)‐308 G/A of TNF‐alpha gene as genetic marker associated with angina pectoris in a sample of Iraqi patients. **Journal of Genetic Engineering and Biotechnology**, 2023.

AHMED, A. A. *et al.* TNF-α − 308 G/A and IFN-γ + 874 A/T gene polymorphisms in Saudi patients with cutaneous leishmaniasis. **BMC Medical Genetics**, v. 21, n. 1, p. 104, dez. 2020.

BARNES, B. J. *et al.* Targeting potential drivers of COVID-19: Neutrophil extracellular traps. **Journal of Experimental Medicine**, v. 217, n. 6, p. 52, 1 jun. 2020.

BERNSTEIN, K. E. *et al.* A Modern Understanding of the Traditional and Nontraditional Biological Functions of Angiotensin-Converting Enzyme. **Pharmacological Reviews**, v. 65, n. 1, p. 1–46, jan. 2013.

CABRERA MARTIMBIANCO, A. L. *et al.* Frequency, signs and symptoms, and criteria adopted for long COVID-19: A systematic review. **International Journal of Clinical Practice**, v. 75, n. 10, out. 2021.

CHAKRAVORTY, S.; HEGDE, M. Inferring the effect of genomic variation in the new era of genomics. **Human Mutation**, v. 39, n. 6, p. 756–773, jun. 2018.

CIOTTI, M. *et al.* The COVID-19 pandemic. **Critical Reviews in Clinical Laboratory Sciences**, v. 57, n. 6, p. 365–388, 17 ago. 2020.

CORIC, V. *et al. GSTP1* and GSTM3 Variant Alleles Affect Susceptibility and Severity of COVID-19. **Frontiers in Molecular Biosciences**, v. 8, p. 747493, 20 dez. 2021.

CUI, J. *et al. GSTP1* and cancer: Expression, methylation, polymorphisms and signaling (Review). **International Journal of Oncology**, 10 fev. 2020.

ELEZKURTAJ, S. *et al.* Causes of death and comorbidities in hospitalized patients with COVID-19. **Scientific Reports**, v. 11, n. 1, p. 4263, 19 fev. 2021.

EL-TAHAN, R. R.; GHONEIM, A. M.; EL-MASHAD, N. TNF-α gene polymorphisms and expression. **SpringerPlus**, v. 5, n. 1, p. 1508, dez. 2016.

FEYDA NURSAL, A. *et al.* TNF-α-308 G/A variant may be associated with bipolar disorder in a Turkish population. **Archives of Clinical Psychiatry (São Paulo)**, v. 47, n. 6, 2020.

GILLILAND, F. D. *et al.* Effects of Glutathione-S-Transferase M1, T1, and P1 on Childhood Lung Function Growth. **American Journal of Respiratory and Critical Care Medicine**, v. 166, n. 5, p. 710–716, set. 2002.

GRASSELLI, G. *et al.* Pathophysiology of COVID-19-associated acute respiratory distress syndrome: a multicentre prospective observational study. **The Lancet Respiratory Medicine**,

v. 8, n. 12, p. 1201–1208, dez. 2020.

GUO, Y. *et al.* Targeting TNF-α for COVID-19: Recent Advanced and Controversies. **Frontiers in Public Health**, v. 10, p. 833967, 11 fev. 2022.

HASHEMI, M. *et al.* Association of Genetic Polymorphisms of Glutathione-S-Transferase Genes (*GSTT1*, *GSTM1*, and *GSTP1*) and Susceptibility to Nonalcoholic Fatty Liver Disease in Zahedan, Southeast Iran. **DNA and Cell Biology**, v. 31, n. 5, p. 672–677, maio 2012.

HWANG, T.-J. *et al.* Loneliness and social isolation during the COVID-19 pandemic. **International Psychogeriatrics**, v. 32, n. 10, p. 1217–1220, out. 2020.

JANIK, E. *et al.* The Emerging Concern and Interest SARS-CoV-2Variants. **Pathogens**, v. 10, n. 6, p. 633, 21 maio. 2021.

KUMAR, R.; SRIVASTAVA, V.; NAND, K. N. The Two Sides of the COVID-19 Pandemic. **COVID**, v. 3, n. 12, p. 1746–1760, 4 dez. 2023.

LAMERS, M. M.; HAAGMANS, B. L. SARS-CoV-2pathogenesis. **Nature Reviews Microbiology**, v. 20, n. 5, p. 270–284, maio. 2022.LI, Q. *et al.* Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus–Infected Pneumonia. **New England Journal of Medicine**, v. 382, n. 13, p. 1199–1207, 26 mar. 2020.

LIMA, C. M. A. DE O. Informações sobre a nova doença coronavírus (COVID-19). **Radiologia Brasileira**, v. 53, n. 2, p. V–VI, abr. 2020.

LOGSDON, G. A.; VOLLGER, M. R.; EICHLER, E. E. Long-read human genome sequencing and its applications. **Nature Reviews Genetics**, v. 21, n. 10, p. 597–614, out. 2020.

MALIK, Y A. “Properties of Coronavirus and SARS-CoV-2.” **The Malaysian journal of pathology,** v. 42, p. 3-11, jan 2020 .

MARKOVIC, M. *et al.* GPX3 Variant Genotype Affects the Risk of Developing Severe Forms of COVID-19. **International Journal of Molecular Sciences**, v. 24, n. 22, p. 16151, 10 nov. 2023. OMS. World Health Organization **Living Guidance for Clinical Management of COVID-19**. World Health Organization,116p, 23 nov. 2021.

MOURA, E. L. D. *et al.* TNF-α-308A>G and IL-10-819C>T polymorphisms as risk factors for cervical cancer: A case-control study, meta-analysis and trial sequential analysis. **Meta Gene**, v. 29, p. 100943, set. 2021.

OMS. World Health Organization **Living Guidance for Clinical Management of COVID-19**. World Health Organization,116p, 23 nov. 2021.

ORLEWSKA, K. *et al.* Association between Glutathione S-Transferases Gene Variants and COVID-19 Severity in Previously Vaccinated and Unvaccinated Polish Patients with Confirmed SARS-CoV-2Infection. **International Journal of Environmental Research and Public Health**, v. 20, n. 4, p. 3752, 20 fev. 2023.

PADULA, M. C. *et al.* Correlation of Tumor Necrosis Factor-α −308G>A Polymorphism with Susceptibility, Clinical Manifestations, and Severity in Behçet Syndrome: Evidences from an Italian Genetic Case–Control Study. **DNA and Cell Biology**, v. 39, n. 7, p. 1104–1110, 1 jul. 2020.

PECORARO, V.; CUCCORESE, M.; TRENTI, T. Genetic polymorphisms of ACE1, ACE2, IFTM3, TMPRSS2 and TNFα genes associated with susceptibility and severity of SARS-CoV-2 infection: a systematic review and meta-analysis. **Clinical and Experimental Medicine**, v. 23, n. 7, p. 3251–3264, 13 abr. 2023.

PIROLA, S.; PATANÈ, L. Biological Mechanisms of Transplacental SARS-COV-2Transmission. Em: DE LUCA, D.; BENACHI, A. (Eds.). **COVID-19 and Perinatology**. Cham: Springer International Publishing, p. 49–62., 2023.

PURWANTY, Eni; PERMATA, Eva Nilam. Analysis of Risk Factors Associated with Survival COVID-19 Confirmed Patients. **Jurnal RSMH Palembang** , v. 1, pág. 240-251, 2023.

SALEH, A. *et al.* Association of TNF-α G-308 a Promoter Polymorphism with the Course and Outcome of COVID-19 Patients. **Immunological Investigations**, v. 51, n. 3, p. 546–557, 3 abr. 2022.

SANTIAGO, A. M. *et al.* TNFA -308G>A and IL10 -1082A>G polymorphisms seem to be predictive biomarkers of chronic HCV infection. **BMC Infectious Diseases**, v. 21, n. 1, p. 1133, dez. 2021.

SCHMIEDEL, B. J. *et al.* Impact of Genetic Polymorphisms on Human Immune Cell Gene Expression. **Cell**, v. 175, n. 6, p. 1701- 1715.e 16, nov. 2018.

SILVA, E. *et al.* **Effect modification by GST and TNF-α polymorphisms in the association between air pollution and lung function in asthmatic children**. [s.l.] Preprints, 10 jul. 2021a.

Secretaria Estadual de Saúde do Paraná - SESA - Centro de Estudos e Pesquisa em Terapia Intensiva. **Protocolo de atendimento de paciente com COVID-19**, 2021. Disponível em : https://www.saude.pr.gov.br/Pagina/Coronavirus-COVID-19. Acesso em:15 nov.2023.

SOTOMAYOR-LUGO, F. *et al*. The role of tumor necrosis factor alpha − 308A > G polymorphism on the clinical states of SARS-CoV-2infection. **Egyptian Journal of Medical Human Genetics**, v. 23, n. 1, p. 55, dez. 2022.

STRUYF, T. *et al.* Signs and symptoms to determine if a patient presenting in primary care or hospital outpatient settings has COVID-19. **Cochrane Database of Systematic Reviews**, v. 2022, n. 5, 20 maio 2022.

THOMAS, P. S. Tumour necrosis factor-α: The role of this multifunctional cytokine in asthma. **Immunology & Cell Biology**, v. 79, n. 2, p. 132–140, abr. 2001.

TOPCUOGLU, N. Public Health Emergency of International Concern: Coronavirus Disease 2019 (COVID-19). **The Open Dentistry Journal**, v. 14, n. 1, p. 71–72, 20 mar. 2020.

TORRES,DA SILVA M. K. *et al.* The Complexity of SARS-CoV-2Infection and the COVID-19 Pandemic. **Frontiers in Microbiology**, v. 13, p. 789882, 10 fev. 2022.

TRUGILO, K. P. *et al.* Polymorphisms in the TGFB1 signal peptide influence human papillomavirus infection and development of cervical lesions. **Medical Microbiology and Immunology**, v. 208, n. 1, p. 49–58, fev. 2019.

TUROŇOVÁ, B. *et al.* In situ structural analysis of SARS-CoV-2spike reveals flexibility mediated by three hinges. **Science**, v. 370, n. 6513, p. 203–208, 9 out. 2020.

VITALE, R. F.; RIBEIRO, F. D. A. Q. O papel do Fator de Necrose Tumoral Alfa (TNF-alfa)

no processo de erosão óssea presente no colesteatoma adquirido da orelha média. **Revista Brasileira de Otorrinolaringologia**, v. 73, n. 1, p. 123–127, fev. 2007.

WANG, J. *et al.* Individual bat virome analysis reveals co-infection and spillover among bats and virus zoonotic potential. **Nature Communications**, v. 14, n. 1, p. 4079, 10 jul. 2023.

WEI, J. *et al.* Why does the spread of COVID-19 vary greatly in different countries? Revealing the efficacy of face masks in epidemic prevention. **Epidemiology and Infection**, v. 149, p. e24, 2021.

WU, F. *et al.* A new coronavirus associated with human respiratory disease in China. **Nature**, v. 579, n. 7798, p. 265–269, 12 mar. 2020.

YANG, Q. *et al.* EPHX1 and *GSTP1* polymorphisms are associated with COPD risk: a systematic review and meta-analysis. **Frontiers in Genetics**, v. 14, p. 85 -- 89, 22 maio. 2023.

5. ARTIGO CIENTÍFICO

GSTP1 variant rs1695 affects susceptibility to severity of COVID-19

ABSTRACT

SARS-CoV-2, the novel coronavirus agent of COVID-19, has spread rapidly worldwide with clinical manifestations ranging from asymptomatic, mild, moderate to severe, and often fatal. Understanding the pathophysiology of SARS-CoV-2 for disease control and clinical management remains a challenge. Factors influencing the development of COVID-19 include comorbidities, age, sex, and lifestyle. Genetic factors are also being investigated and may influence susceptibility and disease progression. The objective of this study was to determine the allele and genotype frequencies of *GSTP1* rs1695 and *TNF* A-308 (rs1800629) in hospitalized patients with COVID-19 and to verify the association of the polymorphisms with severity, comorbidity, and outcome of hospitalization in patients from southern Brazil. A total of 236 patients with COVID-19 who were admitted to a reference hospital in Paraná, Brazil, participated in the study. Blood samples were collected from individuals for genetic characterization using T-ARMS-PCR (polymerase chain reaction-based tetra-primer amplification refractory mutation system) for rs1695 and ARMS-PCR (polymerase chain reaction-based amplification refractory mutation system) for rs1800629. Patient medical records were searched to collect sociodemographic, clinical, and comorbidity data. Bivariate and multivariate analyses, including logistic regression, were then performed. Population was 58.1% men and 41.9% women; 63% had severe and 37% moderate cases of COVID-19; 64% were discharged from hospital and 36% died. The results showed that the ancestral allele was most common in rs1695 (A = 71%) and rs1800629 (G = 61%), and the AA (rs1695) and AG (rs1800629) genotypes were 48.73% and 67.81%, respectively. Regarding rs1695, both A allele (OR = 2.9; p = 0.033) and AG genotype (OR = 3.3; p = 0.022) increased the odds of COVID-19 severity. No significant association was observed for the rs1800629 polymorphism. Also, no association was observed regarding hospital outcome, comorbidities, oxygen saturation, and length of hospital stay. We conclude that both the A allele and AG genotype of *GSTP1* rs1695 are suggestive markers for COVID-19 severity in southern Brazil. No association was found for *TNF* A-308 rs1800629 polymorphism.

Keywords: SARS-CoV-2; Single-nucleotide polymorphism; Glutathione S-transferase; Cytokine; Comorbidity.

INTRODUCTION

SARS-CoV-2, a human coronavirus discovered in December 2019 in the city of Wuhan, Hubei province, China, is the seventh type of coronavirus to infect humans and develop into an atypical pneumonia termed COVID-19 (New Coronavirus Disease) [1,2]. Because of its high transmissibility, SARS-CoV-2 quickly spread to several countries, culminating in a pandemic declared by the World Health Organization (WHO) in March 2020, less than three months after its discovery [3].

Despite the end of the SARS-CoV-2 pandemic in May 2023 [4], the global picture of COVID-19 in February 2024 showed 774 million confirmed cases with a case–fatality rate of 0.90%. The Brazilian Ministry of

Health recorded over 38 million positive cases with a case–fatality rate of 1.8% in February 2024 [5]. The high transmissibility of SARS-CoV-2 and its progression to severe acute respiratory syndrome (SARS) led to respiratory failure requiring intensive care in many cases, resulting in a high mortality rate [6-8].

As with other viral diseases, COVID-19 can be asymptomatic or cause a variety of symptoms [9-11]. The condition of a patient with COVID-19 can be classified as mild, moderate, or severe depending on the clinical manifestations [12]. The oxygen saturation index, combined with warning symptoms such as fever and shortness of breath, was one parameter used to classify disease severity [13]. In addition, predictors of COVID-19 severity such as age, sex, and comorbidities might be related to dysregulation of the immune inflammatory response to viral infection [14, 15]. Another factor that has attracted attention is the progression to severe COVID-19 even in individuals with no history of comorbidities, which raises the hypothesis that the individual genetic profile may be a potential factor in disease severity [16].

SARS-CoV-2 binding to ACE2 (angiotensin-converting enzyme 2) receptors, which are expressed in several human tissues, as well as an exaggerated immune response leading to cytokine storm or cytokine release syndrome (CRS) [17] have been implicated in the pathogenesis of COVID-19. This has been confirmed in autopsies, suggesting an association with acute respiratory distress syndrome (ARDS) [18]. Increased serum levels of proinflammatory cytokines have been observed in patients with severe COVID-19, which may trigger CRS [19].

Tumor necrosis factor alpha (*TNF A*) is a multifunctional, proinflammatory cytokine that belongs to the tumor necrosis factor (TNF) superfamily [20]. *TNF A* is considered one of the most important cytokines associated with inflammatory and immune processes, acting in various tissues to aid the body's defense against invading microorganisms [21]. In addition, CRS also causes cellular and tissue stress, which is also regulated by enzymes of the glutathione S-transferase (GST) family. These enzymes are involved in cellular redox balance, which is significant in the pathobiology of several diseases, including respiratory diseases [22, 23]. GSTs comprise eight classes, with glutathione S-transferase Pi (*GSTP1*) being one of the enzymes active in the lung [16, 23].

Single-nucleotide polymorphisms (SNPs) are the most common form of human genetic variation and are essential for mapping individual genetic traits [24]. The presence of SNPs in genes or promoter regions may be associated with the progression of a particular disease or susceptibility to a pathogen [25, 26]. Both GSTP1 and TNF A have polymorphic regions that can influence the body's immune response [27, 28].

These polymorphic variations in the host genome, including those of the GST and TNF families, have been studied in COVID-19 [16, 29-31]. A study of Polish patients carrying the Ile/Val *GSTP1* (rs1695) genotype showed an almost threefold increased risk of disease severity compared to other genotypes [16]. Conversely, the same genotype (Ile/Val *GSTP1*) was associated with a lower risk of developing COVID-19 in the Serbian population [31]. For the *TNF A*-308 SNP (rs1800629), both the A allele and the AA genotype increased the odds of developing COVID-19 and severity, respectively, in the Egyptian population [32]. In Cubans, individuals with the A allele were more symptomatic; however, the severity of COVID-19 was evident in carriers of the G allele [33].

Although studies have reported results correlating the *GSTP1* (rs1695) and *TNF A*-308 (rs1800629) polymorphisms with the severity progression of COVID-19, the results are contradictory and have been studied in few regions worldwide. To date, there has been no study of these SNPs in Brazilian patients with COVID-19. In addition, including studies with populations of different ethnicities and geographic locations regarding the

relationship of polymorphisms with pathology may guide personalized prognosis of COVID-19.

The present study aimed to determine the allele and genotype frequencies of *GSTP1* (rs1695) and *TNF A*-308 (rs1800629) in hospitalized patients with COVID-19 and to verify the association of SNPs with severity, presence of comorbidity, and hospital outcome in patients from southern Brazil.

MATERIALS AND METHODS

Research population, design of the study and sampling

The present quantitative, descriptive, cross-sectional research was conducted in the city of Francisco Beltrão, southwestern Paraná, Brazil. We enrolled patients who were hospitalized between August 2020 and August 2021, initially suspected and later diagnosed with COVID-19, at the Dr. Walter Alberto Pecóits Southwest Regional Hospital (SRH), a reference hospital for COVID-19 in southwestern Paraná.

Inclusion criteria for study participants were individuals who tested positive for SARS-CoV-2 by standard real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) and were hospitalized for at least one day. Exclusion criteria were those who were suspected but tested negative for SARS-CoV-2 and those who were not hospitalized. The project was approved by the Human Research Ethics Committee (CEP) of Universidade Estadual do Oeste do Paraná, under opinion number no. 4.224.011 and certificate of ethical appreciation no. 31837720.5.0000.0107.

The sample consisted of 236 persons. During hospitalization, a form was filled out with various patient information acquired from medical records, the individual notification form on the Notifiable Diseases Information System, the GAL/Lacen Notifica COVID-19 system, and through the Hospital and Outpatient Management System. Information collected includes age, sex, symptoms, comorbidities, disease severity classification (moderate or severe as per WHO), total hospital length of stay, intensive care unit length of stay, oxygen saturation index (SpO_2), need for noninvasive and invasive mechanical ventilation, hospital discharge, or death.

A 5 ml venous blood sample was drawn from each individual, collected in tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA), homogenized, and placed in a thermal box with ice for laboratory analysis. The samples were then sent to the Laboratory of Molecular Biology and Human Cytogenetics of the State University of Western Paraná (UNIOESTE), where they were stored in a freezer at -20ºC until processing. Blood was collected during the hospitalization process of the individuals by health care workers from the COVID-19 reference sectors of the SRH, who were already collecting material daily for routine laboratory analysis to monitor these patients.

Isolation of genetic material

Total DNA extraction from each individual was performed by using a 200 µl aliquot of the original blood sample according to the QIAamp® DNA Mini Kit Protocol, DNA Purification from Blood or Body Fluids (Spin Protocol) (Qiagen, Germany) and stored in a -20 °C freezer according to the manufacturer's instructions. After DNA extraction, the presence of a segment of approximately 268 bp of the human β-globin gene was detected by using the GH20 (5′-GAAGAGCCAAGGACAGGTAC-3′) and PC04 (5′-CAACTTCATCCACGTTCACC-3′)

primers to verify the presence and quality of DNA samples. The amplification conditions for each reaction were as follows: 190 nM dNTPs, 500 nM of each primer, 2 mM $MgCl_2$, buffer (200 mM Tris-HCL, 500 mM KCl), 1.25 U DNA polymerase (Ludwig™), and approximately 50 ng DNA subjected to a series of cycling steps: 94 °C for 10 minutes; 37 cycles of 94 °C for 1 minute, 55 °C for 1 minute, 72 °C for 1 minute; 72 °C for 10 minutes [34].

Genotyping *GSTP1* (rs1695) and *TNF A*-308 (rs1800629)

The method used to determine the genetic *GSTP1* (rs1695) polymorphism was polymerase chain reaction-based tetra-primer amplification refractory mutation system (T-ARMS-PCR) based on a previous protocol [35]; to determine the *TNF A*-308 (rs1800629) polymorphism, we used polymerase chain reaction-based amplification refractory mutation system (ARMS-PCR) [36].

For rs1695, the following primer pairs were used: forward outer 5'-CAGGTGTCAGGTGAGCTCTGAGCACC-3' and reverse outer 5'-ATAAGGGTGCAGGTTGTGTCTTGTCCCA-3' for the A allele (isoleucine), and forward inner 5'-CGTGGACCTCCTCCGCTGCAAATCCA-3' and reverse Inner 5'-GCTCACATAGTTGGTGTAGATGAGGGATAC-3' for the G allele (valine). The cycling steps included: initial denaturation at 95 °C for 5 minutes, followed by 30 cycles of 95 °C for 35 seconds, 62 °C for 35 seconds, 72 °C for 50 seconds, with a final extension at 72 °C for 10 minutes [35]. The generated amplicons were 233bp for the A allele, 290bp for the G allele, and 467bp for external primers/control band (Figure 1).

For the TNF A-308 SNP (rs1800629), a common forward primer was used for both alleles: Forward 5'-TCTCGGTTTCTTCTCCATCG - 3'; and the reverse primer was 5'-ATAGGTTTGAGGGGCATGA - 3' for the A allele and 5'-ATAGGTTTGAGGGGCATGG - 3' for the G allele. A and G alleles were evaluated separately as they have the same amplicon size (184 bp) [36] (Figure 2). Cycling conditions included an initial denaturation at 95 °C for 2 minutes, followed by 30 cycles of 95 °C for 15 seconds, 65 °C for 50 seconds, 72 °C for 1 minute, with a final extension at 72 °C for 5 minutes [36].

Genotypes of both SNPs were determined by migration of the products on a 2% agarose gel. They were visualized under UV light and subsequently photographed (Figure 1 and 2).

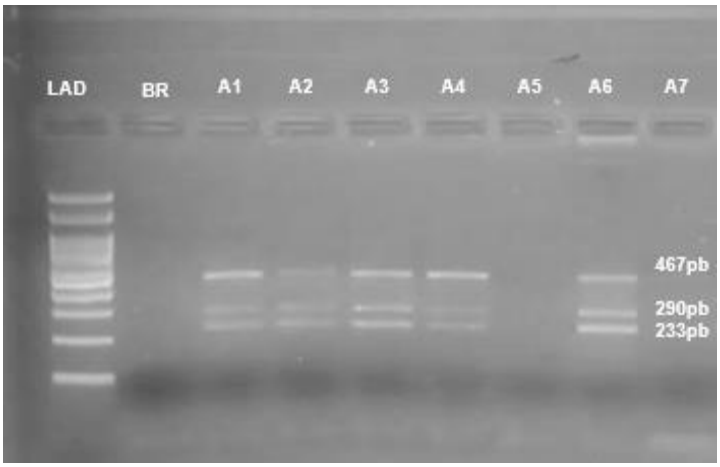


Figure 1. Image of a 2% agarose gel representing the *GSTP1* polymorphism (rs1695). LAD (molecular weight standard of 100 bp); BR (blank or negative control); A1, A2, A3, A4, A5, A6, and A7 are the samples. The molecular weight is 467 bp for the control, 290 bp for the G allele, and 233 bp for the A allele.

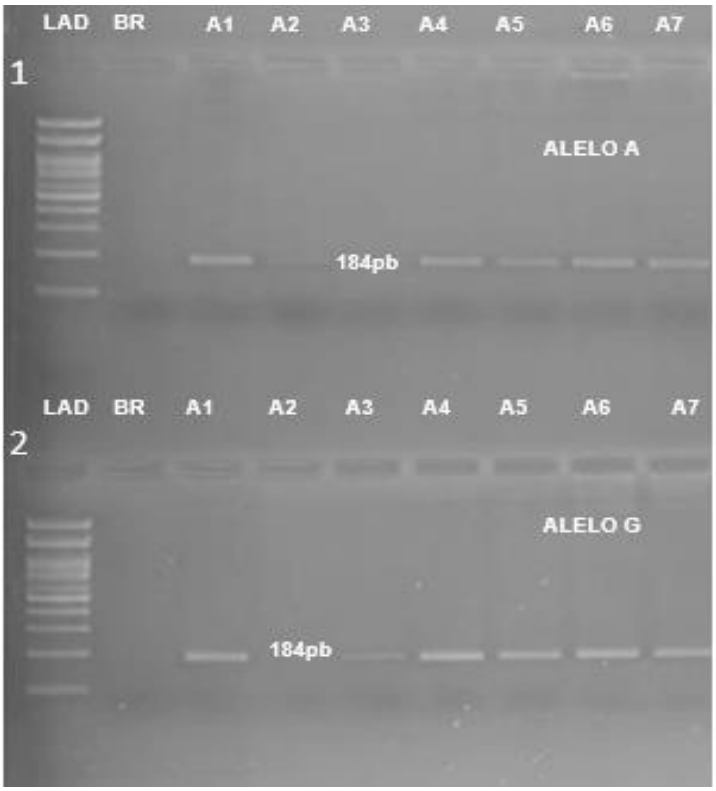


Figure 2. Image of a 2% agarose gel representing the *TNF A*-308 polymorphism (rs1800629). LAD (molecular weight standard of 100 bp); BR (blank or negative control); A1, A2, A3, A4, A5, A6, and A7 are the samples. A allele (1) and G allele (2), both with a fragment size of 184bp.

Statistical analysis

Data were tabulated and analyzed by using the 24.0 Statistical Package for the Social Sciences (SPSS). Continuous variables were tested for normal distribution by means of the Kolmogorov-Smirnov test ($p<0.05$), and the remaining variables were categorized in the same way. Allele and genotype frequencies were determined, and Hardy–Weinberg equilibrium (HWE) was tested using the chi-square test. Chi-square test was also used with Yates continuity correction and Fisher's exact test for comparisons between independent categorical variables (e.g., case severity, outcome of death or hospital discharge, and comorbidities) with SNPs. Independent variables that showed significance less than 0.20 ($p < 0.20$) in the bivariate analyses were subjected to logistic regression using the stepwise method, considering 95% confidence intervals and $p > 0.05$, to determine the odds ratio (OR). To verify the association between genetic polymorphisms and oxygen saturation level and length of hospital stay, Student's t-test was performed, considering $p < 0.05$.

RESULTS

Overall and genotype characteristics of individuals

Study sample included 236 hospitalized patients with COVID-19. The mean age of the patients was 57 years (15–89 years). The proportion of women with COVID-19 was 41.9%, while the proportion of men was 58.1%. Regarding COVID-19 severity, 63% were considered severe and 37% moderate, with hospital discharge

being the most common outcome (64%). With respect to comorbidities, systemic arterial hypertension (SAH) was present in 48.7% of individuals, followed by obesity (45%) and cardiovascular disease (22%). Chronic obstructive pulmonary disease (COPD) and respiratory diseases were observed in less than 6% of hospitalized patients (Table 1).

Table 1. Overall characteristics of the group of hospitalized patients diagnosed with COVID-19 in the city of Francisco Beltrão, state of Paraná, Brazil

Variables	**Participants (n= 236)**	**Frequency (%)**
Age (mean = 57.28 [15–89 ± 16,2] years)		
Sex		
Men	137	58.1
Women	99	41.9
COVID-19 severity		
Severe	149	63.1
Moderate	87	36.9
Outcome		
Death	85	36
Discharge	151	64
Comorbidities		
Systemic arterial hypertension	115	48.7
Obesity	108	45.8
Cardiovascular disease	53	22.5
Diabetes mellitus	38	16.1
Chronic obstructive pulmonary disease	13	5.5
Respiratory tract diseases	12	5.1
Coronary heart disease	11	4.7
Asthma	3	1.3
Kidney diseases	3	1.3

Regarding genetic polymorphisms, 236 individuals were evaluated for *GSTP1* (rs1695) and 233 for *TNF A*-308 (rs1800629) (because the SNP could not be characterized in three patients). For *GSTP1* (rs1695), the frequencies of the A allele (ancestral allele/isoleucine) and the G allele (variant allele/valine) were 71% and 29%, respectively. Regarding the genotypes, the homozygous AA (isoleucine/isoleucine) genotype was prevalent in 48.73%, followed by the heterozygous AG (isoleucine/valine) genotype in 43.64%, and the homozygous G (valine/valine) genotype was found in 7.63% of individuals. For TNF A-308 (rs1800629), the G allele (ancestral) had a frequency of 61% and the A allele (variant) had a frequency of 39%. The genotypic frequencies for TNF A-308 showed that the heterozygous allelic combination (AG) was prevalent (67.81%), followed by homozygous individuals for the G allele (27.47%) and for the A allele (4.72%). Only *GSTP1* showed frequencies in HWE (Table 2).

Table 2. Observed allele and genotype frequencies and Hardy–Weinberg equilibrium (HWE)

SNP	**Genotypes**			*P*	**Alleles**	
***GSTP1* (rs1695)**	AA	GG	AG	= 0.887	A (Ile/ancestral)	G (Val/variant)
Observed number	115	18	103		333	139
Observed frequency	48.73%	7.63%	43.64%		70,6%	29,4%
Expected frequency (HWE)	49.84%	8.64%	41.52%		70.5%	29.5%
***TNF A*-308 (rs1800629)**	AA	GG	AG	< 0.001*	G (ancestral)	A (variant)
Observed number	11	64	158		286	180
Observed frequency	4.72%	27.47%	67.81%		61.4%	38.6%
Expected frequency (HWE)	14.90%	37.70%	47.40%		NS	NS

GSTP1 = Glutathione S-Transferase P1. *TNF A*-308 = Tumor Necrosis Factor Alpha -308. The chi-square test Teste (X^2). To *TNF A-308* (rs1800629) the genotypes frequencies are not in HWE (*p<0,001). NS (not applicable).

Relationship of the polymorphisms *GSTP1* (rs1695) and *TNF A-308* (rs1800629) *with* clinical status, outcome of patients hospitalized and comorbidities

The study showed an association between *GSTP1* (rs1695) and severe COVID-19. The presence of the A allele suggests an almost threefold increased risk of progression to severe disease (OR = 2.9; 95% CI = 1.093 to 7.884; p = 0.033) compared to those with the G allele. Considering genotypic combinations, heterozygous individuals also have more than a threefold increased risk of progression to severe disease (OR = 3.3; 95% CI = 1.185 to 9.375; p = 0.022) compared to homozygous combinations. Regarding the *TNF A*-308 polymorphism (rs1800629), no association was found with clinical variables and outcomes of hospitalized patients with COVID-19 (Table 3).

Table 3. Relationship between *GSTP1* (rs1695) and *TNF A*-308 (rs1800629) genotypes and the clinical status and outcome of patients hospitalized with COVID-19 in the city of Francisco Beltrão, state of Paraná, Brazil

Polymorphisms	**COVID-19 outcome**		*p**	**COVID-19 severity**		*p**	Clinical status		
	Death N (%)	Discharged N (%)		Severe N (%)	Moderate N (%)		*OR*	*p***	*IC95%*
***GSTP1* alleles**									
A (ancestral)	80 (36.7%)	138 (63.3%)	0.616	142 (65.1%)	76 (34.9%)	**0.049**	**2.936**	**0.033**	**(1.093 to 7.884)**
G	40 (33.1%)	81 (66.9%)	0.403	77 (63.6%)	44 (36.4%)	0.977	1 (ref.)		
***GSTP1* genotypes**									
AA	45 (39.1%)	70 (60.9%)	0.579	72 (62.6%)	43 (37.4%)	**0.065**	1.631	0.063	(0.949 to 7.298)
AG	35 (34.0%)	68 (66.0%)		70 (68.0%)	33 (32.0%)		**3.333**	**0.022**	**(1.185 to 9.375)**
GG	5 (27.8%)	13 (72.2%)		7 (38.9%)	11 (61.1%)		1 (ref.)		
***TNF A*-308 alleles**									
G (ancestral)	78 (35.1%)	144 (64.9%)	0.461	141 (63.5%)	81 (36.5%)	0.524			
A	60 (35.5%)	109 (64.5%)	0.960	107 (63.3%)	62 (36.7%)	0.920			
***TNF A*-308 genotypes**									
AA	6 (54.5%)	5 (45.5%)	0.371	6 (54.5%)	5 (45.5%)	0.810			
GG	24 (37.5%)	40 (62.5%)		40 (62.5%)	24 (37.5%)				
AG	54 (34.2%)	104 (65.8%)		101(63.9%)	57 (36.1%)				

GSTP1 = Glutathione S-Transferase P1. *TNF A*-308 = Tumor Necrosis Factor Alpha -308. The significance level (p*) was set at $p < 0.20$ for the chi-square test. p** (95% significance level) for logistic regression. Guidance for total sum of 100% is in the line.

Of the individuals included in the study, 233 had medical records of oxygen saturation (SpO_2) and 222 had records of number of days hospitalized. For these factors, no association was observed with the *GSTP1* (rs1695) and *TNF A*-308 (rs1800629) polymorphisms (Table 4).

Table 4. Association of GSTP1 (rs1695) and TNF-A 308 (rs1800629) with oxygen saturation and length of hospital stay in patients with COVID-19 in the city of Francisco Beltrão, state of Paraná, Brazil

Polymorphism	**O_2 saturation (SO_2%)**		**Length of hospital stay (days)**	
	Mean ± standard deviation	*p**	Mean ± standard deviation	*p**
***GSTP1* alleles**				
A (ancestral)	88.27 ±16.13	0.620	14 ±13.31	0.296
G	88.43 ±16.01		14.35 ±14.03	
***GSTP1* genotypes**				
AA	88.40±15.15	0.619	13.08±12.21	0.219
AG	88.12 ±17.24		15.06±14.45	
GG	90.17±4.95		10.61 ±10.85	
***TNF A*-308 alleles**				
G (ancestral)	88.20±16.01		13.97±13.95	
A	89.35±12.61	0.122	14.44±13.90	0.242
***TNF A*-308 genotypes**				
AA	91.45±3.14	0.234	10.64±6.26	0.206
AG	89.20±13.01		14.73±14.39	
GG	85.70±21.69		12.13±11.14	

GSTP1 = Glutathione S-Transferase P1; *TNF A*-308 = Tumor Necrosis Factor Alpha -308. p* (95% significance level) for the Student's t-test.

The comorbidities evaluated in the population were obesity, DM, SAH, cardiovascular disease, respiratory disease, and COPD. However, no association was found with the SNPs (Table 5). Vascular complications, asthma, and kidney disease were not assessed in relation to the SNPs because of the small number of records in the medical records.

Table 5. Relationship of GSTP1 and *TNF A-308* with comorbidities presented by patients with COVID-19

Polymorphisms	Obesity		*p*	Diabetes		*p*	SAH		*p*
	No N (%)	Yes N (%)		No N (%)	Yes N (%)		No N (%)	Yes N (%)	
***GSTP1* alleles**									
A (ancestral)	111 (52.6%)	100 (47.4%)	1.000	178 (84.4%)	33 (15.6%)	0.318	107 (50.7%)	104 (49.3%)	0.881
G (variant)	66 (57.4%)	49 (42.6%)	0.210	94 (81.7%)	21 (18.3%)	0.615	55 (47.8%)	60 (52.2%)	0.389
***GSTP1* genotypes**									
AA	55 (48.2%)	59 (51.8%)	0.373	97 (85.1%)	17 (14.9%)	0.369	62 (54.4%)	52 (45.6%)	0.484
GG	10 (55.6%)	8 (44.4%)		13 (72.2%)	5 (27.8%)		10 (55.6%)	8 (44.4%)	
AG	56 (57.7%)	41 (42.3%)		81 (83.5%)	16 (16.5%)		45 (46.4%)	52 (53.6%)	
***TNF A*-308 alleles**									
G (ancestral)	113 (52.6%)	102 (47.4%)	0.926	181 (84.2%)	34 (15.8%)	0.428	112 (52.1%)	103 (47.9%)	0.333
A (variant)	86 (52.8%)	77 (47.2%)	1.000	139 (85.3%)	24 (14.7%)	0.409	83 (50.9%)	80 (49.1%)	1.000
***TNF A*-308 genotypes**									
AA	7 (63.6%)	4 (36.4%)	0.751	8 (72.7%)	3 (27.3%)	0.232	3 (27.3%)	8 (72.8%)	0.288
GG	34 (54%)	29 (46%)		50 (79.4%)	13 (20.6%)		32 (50.8)	31 (49.2%)	
AG	79 (52%)	73 (48%)		131(86.2%)	21 (13.8%)		80 (52.6%)	72 (47.4%)	
	Cardiovascular disease			**Respiratory tract disease**			**COPD**		
	No N (%)	Yes N (%)	*p*	No N (%)	Yes N (%)	*p*	No N (%)	Yes N (%)	*p*
***GSTP1* alleles**									
A (ancestral)	165 (78.2%)	46 (21.8%)	0.772	200 (94.8%)	11 (5.2%)	0.675	200 (94.8%)	11 (5.2%)	1.000
G	84 (73%)	31 (27%)	0.120	108 (93.9%)	7 (6.1%)	0.546	109 (94.8%)	6(5.2%)	1.000

Continue

***GSTP1* genotypes**									
AA	94 (82.5%)	20 (17.5%)	0.227	110 (96.5%)	4 (3.5%)	0.426	108 (94.7%)	6 (5.3%)	1.000
GG	13 (72.2%)	5 (27.8%)		18 (100.0%)	0 (0.4%)		92 (94.8%)	5 (5.2%)	
AG	71 (73.2%)	26 (26.8%)		90 (92.8%)	7 (7.2%)		17 (94.4%)	1 (5.6%)	
***TNF A*-308 alleles**									
G (ancestral)	168 (78.1%)	47 (21.9%)	0.568	206 (95.8%)	9 (4.2%)	0.205	205 (95.3%)	10 (4.7%)	0.251
A	127 (77.9%)	36 (22.1%)	0.966	155 (95.1%)	8 (4.9%)	1.000	153 (93.9%)	10 (6.1%)	0.560
***TNF A*-308 genotypes**									
GG	48 (76.2%)	15 (23.8%)	0.418	60 (95.2%)	3 (4.8%)	0.114	61 (96.8%)	2 (3.2%)	0.137
AA	7 (63.6%)	4 (36.4%)		9 (81.8%)	2 (18.2%)		9 (81.8%)	2 (18.2%)	
AG	120 (78.9%)	32 (21.1%)		146 (96.1%)	6 (3.9%)		144 (94.7%)	8 (5.3%)	

SAH = Systemic arterial hypertension; COPD = Chronic obstructive pulmonary disease; *GSTP1* = Glutathione S-Transferase P1. *TNF A*-308 = Tumor necrosis factor alpha-308. Significance level (p) was set at p<0.2 for the Chi-square test. No comorbidity showed an association in the binary logistic regression.

DISCUSSION

As there are numerous factors that affect the progression of COVID-19, investigating the relationship between host genetic aspects and disease progression may contribute to a better and more personalized prognosis. The research results showed that patients with the A allele and heterozygous genotype (AG) for *GSTP1* (rs1695) were more likely to develop severe COVID-19. However, no association with disease was found for *TNF A*-308 (rs1800629).

The *GSTP1* rs1695 polymorphism involves a base substitution in exon 5 (SNP) where guanine replaces adenine at position 313 (A313G) of the gene. Consequently, valine is transcribed instead of the amino acid isoleucine at position 105 of the *GSTP1* enzyme (Ile105Val). This substitution causes a change and reduction of the enzymatic activity with the substrate compared to the ancestral allele, reducing its functionality and thus impairing the immune response of the organism [16, 37].

Regulation of cell proliferation and apoptosis may be associated with the *GSTP1* gene, besides being associated with increased antioxidant activity in the cell [38, 39]. *GSTP1* may have polymorphic regions with functionally relevant variants [40], which have been associated with certain pathologies such as type 2 diabetes mellitus (DM2), chronic kidney disease (CKD), motor neuron disease, and the development of neoplasms [27]. Indeed, the G allele may increase susceptibility to lung cancer, possibly because of the involvement of the enzyme in the regulation of genotoxic substance metabolism. In the Chinese population, the *GSTP1* (rs1695) polymorphism is associated with an increased risk of lung cancer, possibly because of the involvement of the enzyme in the regulation of genotoxic substance metabolism. However, this is not the rule, as SNP variants may be protective for prostate cancer and beneficial for the treatment of leukemia [27].

The *GSTP1* (rs1695) polymorphism has also been studied in comorbidities such as COPD, where the GG genotype (Val/Val) increases the risk of the disease in the Caucasian population (OR = 1.586, 95% CI = 1.210 to 2.080, p = 0.001) [41]. Conversely, another study suggests that the G allele (valine) reduces the risk of developing COVID-19; however, they report a lack of evidence regarding its activity in the SARS-CoV-2 infection process [31].

In parallel with this study, our results suggest that the ancestral A allele, and not the variant G allele of *GSTP1* (rs1695), is a contributing factor in increasing the likelihood of disease severity. A study conducted in vaccinated Polish individuals concluded that the heterozygous genotypic combination of *GSTP1* (Ile/Val) increased the odds of COVID-19 severity by almost three times (OR: 2.75; p = 0.0398) [16]. Although the study found a genotypic effect (AG) rather than an allelic form, it corroborates our findings for the heterozygous profile in the southern Brazilian population, which has 3.33-fold (p = 0.022) higher odds of COVID-19 severity compared to homozygous combinations. Furthermore, the results of our research also showed that the ancestral allelic form increased the odds of disease severity by almost three times (OR: 2.936; p = 0.033).

In contrast, in the Serbian population, the rs1695 AG genotype (Ile/Val) showed a lower predisposition to develop COVID-19 (OR 0.66; 95% CI 0.44 to 0.98; p = 0.042), unless synergistically associated with the *GSTM3* polymorphism, where the risk of developing both COVID-19 and severe disease is increased. Another study [42], also in the Serbian population, found the haplotype generated by the combination of the rs1695 and rs1138272 polymorphisms with the respective G and C alleles reduced the risk of COVID-19 compared to combinations of the ancestral alleles. In contrast, no significant association (OR = 1.02, p = 0.978) was found between rs1695 and COVID-19, nor with the increased oxidative stress caused by the disease in Serbians [43].

Biologically, there may be a plausible explanation for the relationship between the *GSTP1* polymorphism and COVID-19 since among the GSTs, *GSTP1* is one enzyme of the family that is expressed in the lungs, heart, and brain. The presence of the *GSTP1* enzyme, by associating with some kinases, reduces cell death induced by substances such as hydrogen peroxide (H_2O_2). In addition, the enzyme regulates the inflammatory response, suggesting a protective effect against inflammation [44]. However, there is no consensus on the influence of allelic and genotypic forms of *GSTP1* regarding COVID-19 and other diseases. Different results may also be because of ethnic factors, behaviors, and lifestyles of different populations [45].

For the *TNF A*-308 (rs1800629) SNP, no association was found with the outcome of COVID-19. There was also no association with the severity of pathology or the presence of comorbidity in patients infected with SARS-CoV-2. A study conducted in the Serbian population showed that in the inflammatory process of COVID-19 there is an increase in the expression of TNF-α, IL-1β and IL-6 and a progressive worsening of the disease [46, 47]. One study showed that individuals with the A allele (variant) had a higher susceptibility to SARS-CoV-2 infection, with the AA genotype being more susceptible to severity of pathology ($p = 0.001$) [32]. When the TNF A-308 (rs1800629) SNP was studied in Cubans with COVID-19, carriers of the A allele presented a greater variety of symptoms (OR 1.24, 95% CI 0.91-1.70); however, they manifested the severe form of the disease less than those with the G allele (ancestral) (OR 0.96, 95% CI 0.51 to 1.79) [33]. In addition, individuals homozygous for GG are more likely to be asymptomatic compared to other genotypes. The study also shows that this association may vary by geographic region [33]. More recently, the rs1800629 SNP has been defined as a marker to evaluate the clinical conditions of COVID-19 in the Egyptian population [48].

In a meta-analysis [49], the authors concluded that there was no association of rs1800629 with the severity and progression of COVID-19. This result is consistent with the findings of this study and another meta-analysis [50], which confirms the lack of association between rs1800629 and the likelihood of death from COVID-19. In the study population of the present work, the *TNF A*-308 (rs1800629) SNP is not in HWE, which could be a limiting factor to assert its lack of association with COVID-19. However, HWE was observed for rs1695, confirming that patient sampling did not bias the genetic analysis of the population.

Although some studies suggest relevant results depending on the allelic form and genotypic combination for the SNP in individuals affected by the infection, the results are divergent, as in the case of *GSTP1* (rs1695). In the Brazilian population studied in this study, no association was found between rs1800629 and COVID-19 progression, excluding it as a prognostic marker of the disease. Therefore, our main finding confirms that *GSTP1* (rs1695) is indeed an important genetic marker for the severity of COVID-19 in the southern Brazilian population.

The study highlights some limitations, such as the difficulty in analyzing certain clinical data due to the lack of information in medical records amid a pandemic situation. Data on oxidative stress could have complemented the results and broadened the discussion.

CONCLUSION

The results of the present study, previously unexplored in the Brazilian population, revealed an association between the *GSTP1* (rs1695) polymorphism and COVID-19, both for the ancestral allele (A) and its heterozygous combination (AG), defining them as suggestive markers of COVID-19 severity in the southern Brazilian population. However, the *TNF A*-308 (rs1800629) polymorphism has not been characterized as a genetic

marker for disease prognosis in the Brazilian population.

Acknowledgements

The Hospital Regional do Sudoeste do Paraná – Walter Alberto Pecoits and Hospital São Francisco where the collections took place. The *Universidade Estadual do Oeste do Paraná* that guaranteed access to the Molecular Biology laboratory where the samples were processed. The study group at the Molecular Biology and Cytogenetics Laboratory of the *Universidade Estadual do Oeste do Paraná*, Francisco Beltrão campus.

Statements and Declarations

Data availability Statement

Data supporting the findings of this study are available from the corresponding author.

Compliance with ethical standards

The present study was approved by the Ethics Committee in Human Research of Universidade Estadual do Oeste Paraná and the National Committee on Ethics in Research, with legal opinion number 4.224.011 and CAAE: 31837720.5.0000.0107.

Conflict of interest

All authors declare that they have no conflict to interest.

Author Contribution

Conceptualization: P.S.A.V and L.C.L. Methodology: P.S.A.V, M.B.N, F.R.T, A.G.N.A, L.E.D.F and L.C.L. Formal analysis and investigation: P.S.A.V, M.B.N, F.R.T, and L.C.L. Writing - original draft preparation: P.S.A.V, M.B.N, F.R.T, C.R.P, and L.C.L. Writing - review and editing: P.S.A.V, M.B.N, F.R.T, C.R.P, and L.C.L. Resources: A.G.N.A, L.E.D.F, and L.C.L. Supervision: P.S.A.V, M.B.N, F.R.T, and L.C.L.

Consent to Participate

All authors informed consent.

Consent to Publish

All authors approved the version to be published and agree to be accountable for all aspects of the work in ensuring related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG et al (2020) A new coronavirus associated with human respiratory disease in China. Nature, 579(7798): 265–269. https://doi.org/10.1038/s41586-020-2008-3
2. Wang, J, Pan Y, Yang L, Yang W, Lv K, Luo C et al (2023) Individual bat virome analysis reveals co-infection and spillover among bats and virus zoonotic potential. Nature Communications, 14(1):4079. https://doi.org/10.1038/s41467-023-39835-1

3. Topcuoglu N (2020) Public Health Emergency of International Concern: Coronavirus Disease 2019 (COVID-19). The Open Dentistry Journal, 14(1):71–72. https://doi.org/10.2174/1874210602014010071
4. Pan American Health Organization. WHO declares end of Public Health Emergency of International Concern regarding COVID-19 - PAHO/WHO | Pan American Health Organization. Available at: https://www.paho.org/pt/noticias/5-5-2023-oms-declara-fim-da-emergencia-saude-publica-importancia-internacional-referente . Accessed on: 2 Oct. 2023.Landstra CP, & de Koning EJP (2021) COVID-19 and Diabetes: Understanding the Interrelationship and Risks for a Severe Course. Frontiers in Endocrinology, 12, 649525. https://doi.org/10.3389/fendo.2021.649525
5. State Department of Health of Paraná - SESA - Center for Studies and Research in Intensive Care. COVID-19 patient care protocol, 2021. Available at: https://www.saude.pr.gov.br/Pagina/Coronavirus-COVID-19. Accessed on:15 Nov. 2023.
6. Ciotti M, Ciccozzi M, Terrinoni A, Jiang WC, Wang CB, Bernardini S (2020) The COVID-19 pandemic. Critical Reviews in Clinical Laboratory Sciences, 57(6):365–388. https://doi.org/10.1080/10408363.2020.1783198
7. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y et al (2020) Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus–Infected Pneumonia. New England Journal of Medicine, 382(13):1199–1207. https://doi.org/10.1056/NEJMoa2001316
8. Grasselli G, Tonetti T, Protti A, Langer T, Girardis M, Bellani, G et al (2020) Pathophysiology of COVID-19-associated acute respiratory distress syndrome: A multicentre prospective observational study. The Lancet Respiratory Medicine, 8(12): 1201–1208. https://doi.org/10.1016/S2213-2600(20)30370-2
9. Lima CMAO (2020) Information about the new coronavirus disease (COVID-19). Radiologia Brasileira, 53(2), V–VI. https://doi.org/10.1590/0100-3984.2020.53.2e1
10. Cabrera Martimbianco AL, Pacheco RL, Bagattini ÂM, Riera R (2021) Frequency, signs and symptoms, and criteria adopted for long COVID-19: A systematic review. International Journal of Clinical Practice, 75(10). https://doi.org/10.1111/ijcp.14357
11. Struyf T, Deeks JJ, Dinnes J, Takwoingi Y, Davenport C, Leeflang MM et al (2020) Signs and symptoms to determine if a patient presenting in primary care or hospital outpatient settings has COVID-19 disease. Cochrane Database of Systematic Reviews. 7;7(7):CD013665.https://doi.org/10.1002/14651858.CD013665
12. Elezkurtaj S, Greuel S, Ihlow J, Michaelis EG, Bischoff P, Kunze CA et al (2021) Causes of death and comorbidities in hospitalized patients with COVID-19. Scientific Reports, 11(1):4263. https://doi.org/10.1038/s41598-021-82862-5
13. WHO. World Health Organization Living Guidance for Clinical Management of COVID-19. World Health Organization,116p, 23 nov. 2021.
14. Torres MKS, Bichara CDA, Almeida MNS, Vallinoto MC, Queiroz MAF, Vallinoto IM et al (2022) The Complexity of SARS-CoV-2 Infection and the COVID-19 Pandemic. Frontiers in Microbiology, 13:789882. https://doi.org/10.3389/fmicb.2022.789882
15. Purwanty E, Permata EM (2023) Analysis of Risk Factors Associated with Survival COVID-19 Confirmed Patients. Jurnal RSMH Palembang. 1:240-251. https://doi.org/10.37275/JRP.v4i1.41
16. Orlewska K, Klusek J, Zarębska-Michaluk D, Kocańda K. Oblap R, Cedro A et al(2023) Association between Glutathione S-Transferases Gene Variants and COVID-19 Severity in Previously Vaccinated and Unvaccinated Polish Patients with Confirmed SARS-CoV-2 Infection. International Journal of Environmental Research and Public Health, 20(4):3752. https://doi.org/10.3390/ijerph20043752
17. Pirola S, Patanè L (2023) Biological Mechanisms of Transplacental SARS-COV-2 Transmission. Em D. De Luca & A. Benachi (Orgs.), COVID-19 and Perinatology (p. 49–62). Springer International Publishing. https://doi.org/10.1007/978-3-031-29136-4_6
18. Barnes BJ, Adrover JM, Baxter-Stoltzfus A, Borczuk A, Cools-Lartigue J, Crawford JM et al (2020) Targeting potential drivers of COVID-19: Neutrophil extracellular traps. Journal of Experimental Medicine, 217(6):e20200652. https://doi.org/10.1084/jem.20200652
19. Guo Y, Hu K, Li Y, Lu C, Ling K, Cai C et al (2022) Targeting TNF-α for COVID-19: Recent Advanced and Controversies. Frontiers in Public Health, 10:833967. https://doi.org/10.3389/fpubh.2022.833967
20. Thomas PS (2001) Tumour necrosis factor-α: The role of this multifunctional cytokine in asthma. Immunology & Cell Biology, 79(2):132–140. https://doi.org/10.1046/j.1440-1711.2001.00980.x
21. Vitale RF, Ribeiro FAQ (2007) O papel do Fator de Necrose Tumoral Alfa (TNF-alfa) no processo de erosão óssea presente no colesteatoma adquirido da orelha média. Revista Brasileira de Otorrinolaringologia, 73(1):123–127. https://doi.org/10.1590/S0034-72992007000100020
22. Silva E, Perini J, Silveira I, Lopes L, Cortes T, Oliveira B et al (2021). Effect modification by GST and TNF-α polymorphisms in the association between air pollution and lung function in asthmatic children. https://doi.org/10.22541/au.162593049.90437109/v1

23. Gilliland FD, Gauderman WJ, Vora H, Rappaport E, Dubeau L (2002) Effects of Glutathione-S-Transferase M1, T1, and P1 on Childhood Lung Function Growth. American Journal of Respiratory and Critical Care Medicine, 166(5):710–716. https://doi.org/10.1164/rccm.2112065
24. Chakravorty S, Ankala A, Hegde MR (2019) Implementation of Exome Sequencing Assay. Em G. J. Netto & K. L. Kaul (Orgs.), Genomic Applications in Pathology. Springer International Publishing. 203–217. https://doi.org/10.1007/978-3-319-96830-8_17
25. Logsdon GA, Vollger MR, Eichler EE (2020) Long-read human genome sequencing and its applications. Nature Reviews Genetics, 21(10):597–614. https://doi.org/10.1038/s41576-020-0236-x
26. Schmiedel, B. J., Singh, D., Madrigal, A., Valdovino-Gonzalez, A. G., White, B. M., Zapardiel-Gonzalo, J., Ha, B., Altay, G., Greenbaum, J. A., McVicker, G., Seumois, G., Rao, A., Kronenberg, M., Peters, B., & Vijayanand, P. (2018). Impact of Genetic Polymorphisms on Human Immune Cell Gene Expression. Cell, 175(6):1701-1715.e16. https://doi.org/10.1016/j.cell.2018.10.022
27. Cui J, Li G, Yin J, Li L, Tan Y, Wei H et al (2020) GSTP1 and cancer: Expression, methylation, polymorphisms and signaling (Review) International Journal of Oncology. https://doi.org/10.3892/ijo.2020.4979
28. Abdulfattah SY, Samawi FT (2023) Estimating the role of single-nucleotide polymorphism (rs1800629)-308 G/A of TNF-alpha gene as genetic marker associated with angina pectoris in a sample of Iraqi patients. Journal of Genetic Engineering and Biotechnology, 21(1):2. https://doi.org/10.1186/s43141-022-00454-w
29. Padula MC, Leccese P, Lascaro N, Radice RP, Limongi AR, Sorrento GG et al (2020) Correlation of Tumor Necrosis Factor-α −308G>A Polymorphism with Susceptibility, Clinical Manifestations, and Severity in Behçet Syndrome: Evidences from an Italian Genetic Case–Control Study. DNA and Cell Biology, 39(7):1104–1110. https://doi.org/10.1089/dna.2020.5361
30. Abbas M, Verma S, Verma S, Siddiqui S, Khan FH, Raza ST et al (2021) Association of GSTM1 and GSTT1 gene polymorphisms with COVID-19 susceptibility and its outcome. Journal of Medical Virology, 93(9):5446–5451. https://doi.org/10.1002/jmv.27076
31. Coric V, Milosevic I, Djukic T, Bukumiric Z, Savic-Radojevic A, Matic M et al (2021). GSTP1 and GSTM3 Variant Alleles Affect Susceptibility and Severity of COVID-19. Frontiers in Molecular Biosciences, 8:747493. https://doi.org/10.3389/fmolb.2021.747493
32. Saleh A, Sultan A, Elashry MA, Farag A, Mortada MI, Ghannam MA et al (2022) Association of TNF-α G-308 a Promoter Polymorphism with the Course and Outcome of COVID-19 Patients. Immunological Investigations, 51(3):546–557. https://doi.org/10.1080/08820139.2020.1851709
33. Sotomayor-Lugo F, Alemañy-Díaz Perera C, Roblejo-Balbuena H, Zúñiga-Rosales Y, Monzón-Benítez G, Suárez-Besil B et al (2022) The role of tumor necrosis factor alpha – 308A > G polymorphism on the clinical states of SARS-CoV-2 infection. Egyptian Journal of Medical Human Genetics, 23(1):55. https://doi.org/10.1186/s43042-022-00274-0
34. Trugilo KP, Cebinelli GCM, Berti FCB, Okuyama NCM, Cezar SF, Sena MM, et al (2019) Polymorphisms in the TGFB1 signal peptide influence human papillomavirus infection and development of cervical lesions. Med Microbiol Immunol 208(1):49–58. https://doi.org/10.1007/s00430-018-0557-y
35. Hashemi M, Eskandari-Nasab E, Fazaeli A, Bahari A, Hashemzehi NA, Shafieipour S, et al (2012) Association of Genetic Polymorphisms of Glutathione-S-Transferase Genes (GSTT1 , GSTM1 , and GSTP1) and Susceptibility to Nonalcoholic Fatty Liver Disease in Zahedan, Southeast Iran. DNA and Cell Biology 31(5):672–7. https://doi.org/10.1089/dna.2011.1343
36. Ahmed AA, Rasheed Z, Salem T, Al-Dhubaibi MS, Al Robaee AA, Alzolibani AA (2020) TNF-α −308 G/A and IFN-γ + 874 A/T gene polymorphisms in Saudi patients with cutaneous leishmaniasis. BMC Medical Genetics, 21(1):104. https://doi.org/10.1186/s12881-020-01043-9
37. Singh HO, Lata S, Angadi M, Bapat S, Pawar J, Nema V et al (2017) Impact of GSTM1, GSTT1 and GSTP1 gene polymorphism and risk of ARV-associated hepatotoxicity in HIV-infected individuals and its modulation. The Pharmacogenomics Journal, 17(1):53–60. https://doi.org/10.1038/tpj.2015.88
38. Holley SL, Fryer AA, Haycock JW, Grubb SEW, Strange RC, Hoban, PR (2007) Differential effects of glutathione S-transferase pi (GSTP1) haplotypes on cell proliferation and apoptosis. Carcinogenesis, 28(11):2268–2273. https://doi.org/10.1093/carcin/bgm135
39. Reszka E, Jabłonowski Z, Wieczorek E, Gromadzińska J, Sosnowski M, Wąsowicz W (2011) GSTP1 mRNA expression in human circulating blood leukocytes is associated with GSTP1 genetic polymorphism. *Clinical Biochemistry*, *44*(13):1153–1155. https://doi.org/10.1016/j.clinbiochem.2011.05.024
40. Saadat M (2017) Evaluation of glutathione S-transferase P1 (GSTP1) Ile105Val polymorphism and susceptibility to type 2 diabetes mellitus, a meta-analysis. EXCLI Journal; 16:Doc1188; ISSN 1611-2156. https://doi.org/10.17179/EXCLI2017-828

41. Yang Q, Huang W, Yin D, Zhang L, Gao Y, Tong J et al (2023) EPHX1 and GSTP1 polymorphisms are associated with COPD risk: A systematic review and meta-analysis. Frontiers in Genetics, 14:1128985. https://doi.org/10.3389/fgene.2023.1128985
42. Jerotic D, Ranin J, Bukumiric Z, Djukic T, Coric V, Savic-Radojevic A et al (2022). *SOD2* rs4880 and GPX1 rs1050450 polymorphisms do not confer risk of COVID-19, but influence inflammation or coagulation parameters in Serbian cohort. Redox Report, 27(1):85–91. https://doi.org/10.1080/13510002.2022.2057707
43. Markovic M, Ranin J, Bukumiric Z et al (2023) GPX3 Variant Genotype Affects the Risk of Developing Severe Forms of COVID-19. International Journal of Molecular Sciences, 24(22):16151. https://doi.org/10.3390/ijms242216151
44. Zhou Y, Cao X, Yang Y, Wang J, Yang W, Ben P et al (2018) Glutathione S-Transferase Pi Prevents Sepsis-Related High Mobility Group Box-1 Protein Translocation and Release. Frontiers in Immunology, 9:268. https://doi.org/10.3389/fimmu.2018.00268
45. van de Wetering C, Elko E, Berg M, Schiffers CHJ, Stylianidis V, van den Berge M et al (2021). Glutathione S-transferases and their implications in the lung diseases asthma and chronic obstructive pulmonary disease: Early life susceptibility? Redox Biology, 43:101995. https://doi.org/10.1016/j.redox.2021.101995
46. Manik M, Singh RK (2022) Role of toll-like receptors in modulation of cytokine storm signaling in SARS-CoV-2-induced COVID-19. Journal of Medical Virology, 94(3):869–877. https://doi.org/10.1002/jmv.27405
47. Yarlagadda LC, Ghosh D, Basak U, Senapati M, Das M, Ghosh R (2023) Post-COVID-19 Cardiovascular Sequelae and Myocarditis. J Assoc Physicians India. 71(6):11-12. https://doi.org/10.5005/japi-11001-0256.
48. Eldesouki RE, Kishk RM, Abd El-Fadeal NM, Mahran RI, Kamel N, Riad E, et al (2024) Association of IL-10–592 C > A /-1082 A > G and the TNFα -308 G > A with susceptibility to COVID-19 and clinical outcomes. BMC Medical Genomics, 17(1):40. https://doi.org/10.1186/s12920-023-01793-4
49. Pecoraro V, Cuccorese M, Trenti T (2023) Genetic polymorphisms of ACE1, ACE2, IFTM3, TMPRSS2 and TNFα genes associated with susceptibility and severity of SARS-CoV-2 infection: A systematic review and meta-analysis. Clinical and Experimental Medicine, 23(7):3251–3264. https://doi.org/10.1007/s10238-023-01038-9
50. Araújo JLF, Menezes D, Aguiar RS, Souza RP (2022) IFITM3, FURIN, ACE1, and TNF-α Genetic Association With COVID-19 Outcomes: Systematic Review and Meta-Analysis. Frontiers in Genetics, 13:775246. https://doi.org/10.3389/fgene.2022.775246

6. ANEXOS

UNIOESTE - UNIVERSIDADE ESTADUAL DO OESTE DO PARANÁ

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: CARACTERIZAÇÃO EPIDEMIOLÓGICA E CLÍNICA DA POPULAÇÃO EXPOSTA A COVID-19: ESTUDO TRANSVERSAL

Pesquisador: Lirane Elize Defante Ferreto

Área Temática:

Versão: 3

CAAE: 31837720.5.0000.0107

Instituição Proponente: UNIVERSIDADE ESTADUAL DO OESTE DO PARANA

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.224.011

Apresentação do Projeto:

Título da Pesquisa: CARACTERIZAÇÃO EPIDEMIOLÓGICA E CLÍNICA DA POPULAÇÃO EXPOSTA A COVID-19: ESTUDO TRANSVERSAL

Pesquisador Responsável: Lirane Elize Defante Ferreto

Área Temática:

Versão: 3

CAAE: 31837720.5.0000.0107

Submetido em: 18/08/2020

Instituição Proponente: UNIVERSIDADE ESTADUAL DO OESTE DO PARANA

Situação da Versão do Projeto: Em relatoria

Objetivo da Pesquisa:

Submissão Emenda n. 025 anexa na PB

Avaliação dos Riscos e Benefícios:

Descrito Anteriormente

Comentários e Considerações sobre a Pesquisa:

Descrito Anteriormente

Considerações sobre os Termos de apresentação obrigatória:

Descrito Anteriormente

Endereço: RUA UNIVERSITARIA 2069
Bairro: UNIVERSITARIO **CEP:** 85.819-110
UF: PR **Município:** CASCAVEL
Telefone: (45)3220-3092 **E-mail:** cep.prppg@uniloeste.br

Conclusões ou Pendências e Lista de Inadequações:

Emenda aprovada

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1614676_E2.pdf	18/08/2020 17:29:28		Aceito
Declaração de Instituição e Infraestrutura	laboratoriodebiologiamolecular.pdf	18/08/2020 11:58:57	Lirane Elize Defante Ferreto	Aceito
Outros	memorando025cep.pdf	18/08/2020 11:54:27	Lirane Elize Defante Ferreto	Aceito
Projeto Detalhado / Brochura Investigador	COVID19FB2alterado.pdf	18/08/2020 11:51:45	Lirane Elize Defante Ferreto	Aceito
Outros	scan0008.pdf	11/06/2020 13:40:19	Lirane Elize Defante Ferreto	Aceito
Brochura Pesquisa	COVID19FB2.pdf	11/06/2020 13:22:51	Lirane Elize Defante Ferreto	Aceito
Declaração de Instituição e Infraestrutura	scan0006.pdf	11/06/2020 13:19:55	Lirane Elize Defante Ferreto	Aceito
Declaração de Instituição e Infraestrutura	Policlinica.jpeg	11/06/2020 13:09:24	Lirane Elize Defante Ferreto	Aceito
Declaração de Pesquisadores	pesquisadores.png	15/05/2020 15:04:47	Lirane Elize Defante Ferreto	Aceito
Outros	DOCUMENTOINFORMATIVO.pdf	15/05/2020 11:50:46	Lirane Elize Defante Ferreto	Aceito
Projeto Detalhado / Brochura Investigador	COVID19FB.pdf	15/05/2020 11:48:36	Lirane Elize Defante Ferreto	Aceito
Folha de Rosto	folharosto.pdf	15/05/2020 11:22:08	Lirane Elize Defante Ferreto	Aceito
Declaração de Instituição e Infraestrutura	20200514184751956.pdf	14/05/2020 18:07:42	Lirane Elize Defante Ferreto	Aceito
Outros	FORMULARIOCOLETACOVID19.pdf	14/05/2020 18:06:43	Lirane Elize Defante Ferreto	Aceito
Outros	20200514175721086.pdf	14/05/2020 17:55:12	Lirane Elize Defante Ferreto	Aceito

Endereço: RUA UNIVERSITARIA 2069
Bairro: UNIVERSITARIO **CEP:** 85.819-110
UF: PR **Município:** CASCAVEL
Telefone: (45)3220-3092 **E-mail:** cep.prppg@unioeste.br

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

CASCAVEL, 19 de Agosto de 2020

Assinado por:
Dartel Ferrari de Lima
(Coordenador(a))

Endereço: RUA UNIVERSITARIA 2069
Bairro: UNIVERSITARIO **CEP:** 85.819-110
UF: PR **Município:** CASCAVEL
Telefone: (45)3220-3092 **E-mail:** cep.prppg@unioeste.br

Normas da revista "Archives of Virology"
Link do Guidelines: https://link.springer.com/journal/705/submission-guidelines

Instructions for Authors

Authorship Policy

Authorship should incorporate and should be restricted to those who have contributed substantially to the work in one or more of the following categories:
• Conceived of or designed study
• Performed research
• Analyzed data
• Contributed new methods or models
• Wrote the paper

Back to top

Types of papers

Archives of Virology publishes Original Articles, Brief Reports, Brief Reviews, Annotated Sequence Records, and Special Issues.
Please submit your manuscript in the correct format for each article type. Manuscripts in an incorrect format will be immediately returned to the authors for reformatting.
Every submitted manuscript will be examined by the software "ithenticate" for evidence of plagiarism. Manuscripts that are flagged by this software will be returned to the authors for rewriting.
The journal will accept manuscripts with high-quality electron micrographs of viruses representing new taxa. Genomic variants and papers without proof of novelty will not be considered for peer review. Electron micrographs of poor contrast, unsharp and of small size, as well as micrographs without an indication of dimensions or of the methods used will be rejected. Complete particle descriptions, comparisons with viruses of the same host groups, discussion of relationships and evidence of novelty must be presented.

Original Articles

Papers describing sequences only will only be considered for publication as "Original Articles" or "Brief Reports" if the genomic organization derived from the nucleotide sequences determined differs fundamentally from those of typical members of the virus genus/family. Preferably, the biological significance and function of certain sequence differences should also have been experimentally addressed.
If a manuscript only describes the **c•o•m•p•l•e•t•e** sequence of a virus for which no or only very limited sequence information is available, the manuscript can be considered for submission in the format of an Annotated Sequence Record (see link 'Annotated Sequence Records')". To facilitate a thorough review of any sequence-based manuscript, sequences generated by the author(s) and described in the manuscript **must** be either available from GenBank or some other public database, or provided as FASTA (or similar) files together with the submitted manuscript.
Original articles should not exceed 20 pages when printed (a manuscript pages with 3600 characters usually results in one printed page).
Their content should be arranged as follows:

- Title Page (see below)
- Abstract (see below)
- Introduction: The Introduction should supply sufficient background information to establish the context of the present study—it should allow the reader to see the rationale for the present work and to understand and evaluate present results—it should not be too general, nor should it take the form of an exhaustive review of the subject. The Introduction should usually end with one or two sentences that capture the essence of the article: e.g., “In this paper we report the discovery of …”
- Materials and methods: The Materials and methods section should provide sufficient information to permit the work to be repeated. For commonly used methods, a brief description (to avoid constant need to refer to previous publications) and citation of a reference are sufficient. New methods should be described completely, giving sources of unusual chemicals, equipment, and supplies. When large numbers of viruses, mutants, etc., are used in a study, a table may be used to identify sources, properties, etc.
- Results: The Results section should include the outcome of experiments; extensive interpretations of experimental data should be reserved for the Discussion section. Data should be presented in text, tables, or figures—the same data should not be repeated in two or three forms.
- Discussion: The Discussion section should not merely restate the experimental results and immediate conclusions. It should be constructive, interpretive, analytical, and it should establish the relationship between the results obtained and previously published work. It should note problems, such as conflicts with the ideas and data of others, and it should indicate the value of the results for future research.
- Acknowledgments: Acknowledgments of personal assistance and financial support should be stated in concise terms.
- References (see below)

Brief Reports

Papers describing sequences only will only be considered for publication as “Original Articles” or “Brief Reports” if the genomic organization derived from the nucleotide sequences determined differs fundamentally from those of typical members of the virus genus/family. Preferably, the biological significance and function of certain sequence differences should also have been experimentally addressed.
If a manuscript only describes the **complete** sequence of a virus for which no or only very limited sequence information is available, the manuscript can be considered for submission in the format of an Annotated Sequence Record (see link ‘Annotated Sequence Records’)”. To facilitate a thorough review of any sequence-based manuscript, sequences generated by the author(s) and described in the manuscript **must** be either available from GenBank or some other public database, or provided as FASTA (or similar) files together with the submitted manuscript.
Brief Reports are intended for the presentation of observations that do not warrant a full-length article—they are not meant for preliminary communication of incomplete studies.

- They should not exceed six pages (21000 characters incl. spaces) when printed. This should include all the text, i. e. short Abstract (no more than 100 words), Acknowledgements, References and legends. Division of the text by headings of sections should be omitted, but the general sequence of introduction, materials and methods, results, and discussion may be generally maintained. References should be cited in the same way as in full-length articles. In addition to the text, a maximum of 3 figures or 3 Tables (any combination of 3 such items) can be included.

Annotated Sequence Records

- Annotated Sequence Record papers are intended to draw attention to the availability of **c•o•m•p•l•e•t•e** viral sequences that are appreciably different from those of known sequenced isolates. Currently, we welcome the molecular description of isolate(s) in the format of an Annotated Sequence Record only if (i) the complete genome sequence of a new or established member of a virus genus is reported for the first time and (ii) the isolate of a virus species under study has unusual molecular features (in terms of differences in sequence identity, genome organization or recombination) and/or differs strikingly from other isolates of the virus in biological properties. Sequences that do not differ from already deposited or published type (reference) sequences by more than random mutations will not be accepted for publication. Sequences generated by high throughput sequencing must be confirmed by substantial RACE data. Sequences derived from pooled samples of biological specimens will not be accepted for publication. Genome sequences generated by data-mining will not be accepted.
- Division of the text by headings of sections should be omitted.
- To facilitate a thorough review of any sequence-based manuscript, sequences generated by the author(s) and described in the manuscript must be either available from GenBank or some other public database, or provided as FASTA (or similar) files together with the submitted manuscript.
- These papers should not exceed two pages in length, when printed (which equals four manuscript pages with 1800 characters each [incl. spaces], including references and figure legends, but excluding the title page and tables) and should not have more than 2 figures or tables.
- The report should give information on the provenance of the virus material (isolated by whom, when and where; together with a reference if available), a reference to the sequence (accession number), an annotated diagram of the sequence information (ORFs, promoters, control sequences etc.), some biological information (host range, pathogenicity, etc.) and the justification (i.e. a biological reason that can be derived) for considering why the material is different from previously published isolates.
- Submissions for annotated sequence reports containing only the isolation of a bacteriophage and its genome sequence will no longer be accepted. Such reports must also include a thorough characterisation of the bacteriophage (e.g. transmission electron micrograph (TEM), host spectrum, kinetics of replication, etc) and thus qualify as a brief report or an original article.

Review

Reviews are intended to draw together important information from recent publications on subjects of broad interest. They are meant to provide a venue for critical examination and considered opinion of such information.

- Reviews are not meant to be encyclopedic, and should not exceed 20 pages when printed. Reviews may contain figures and tables. References should be cited in the same way as in full-length articles. It is recommended that authors contact a member of the Editorial Board beforehand to determine if a proposed review is likely to be suitable for publication.

Special Issues

- Special issues of Archives of Virology are published to record the proceedings of meetings, symposia, conferences, and congresses on various virologic topics, special issues are also published to record multi-authored treatises and reviews of large, complex virologic topics. In general, special issues are of similar size and page format as the regular issues of Archives of Virology; the number of pages per issue is limited to 240 pages. The Archives of Virology provides full and flexible publishing and

marketing services, in timely fashion. Individuals who are organizing a meeting, symposium, conference, or congress, and individuals who would like to organize the writing and publication of a treatise or large review are invited to communicate directly with the Special Issues Editor for further information.

Virology Division News:

Papers published under the rubric Virology Division News (VDN) should describe news and developments that are of interest to the Virology Community, including papers on virus taxonomy, classification, and nomenclature, as well as minutes of IUMS or ICTV committees and obituaries of prominent virologists.
If the authors suggest or discuss novel taxa, the authors should include the following Disclaimer to the article:

- The taxonomic changes suggested/proposed/described here

  (i) have not been endorsed by the ICTV Executive Committee,
  (ii) may differ from any new taxonomy that is ultimately approved by the ICTV, and
  (iii) is presented for discussion only but has no official standing.

Further and if applicable, the authors should add the following sentence: “this article is related to an ongoing taxonomic proposal, submitted to the ICTV but not yet accepted at the time of submission."

Back to top

Editorial procedure

Disclaimers: Statements disclaiming governmental or any other type of approval or endorsement will be deleted by the publisher.

Back to top

Manuscript Submission

Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

Permissions

Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) for both the print and online format and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

Online Submission

Please follow the hyperlink “Submit manuscript” and upload all of your manuscript files following the instructions given on the screen.

Source Files

Please ensure you provide all relevant editable source files at every submission and revision. Failing to submit a complete set of editable source files will result in your article not being considered for review. For your manuscript text please always submit in common word processing formats such as .docx or LaTeX.

Please note:

During the online submission process, authors will be prompted for classifications used by the journal to assign editors and reviewers. These classifications should always contain at least one virus family to support the assignment process.
Authors submitting a manuscript should suggest at least two potential reviewers who are acknowledged to have expertise in the subject of the work. These persons must not be or have been members of the institution(s) of the authors or have been associated with them. Being associated in this regard is referring to authors and suggested reviewers publishing together within the last two years.
The current affiliation e-mail address (i.e. hotmail, gmail, yahoo, addresses are not acceptable) as well as an institutional web page address and area of expertise must be provided for each person suggested. Archives of Virology will use such recommended reviewers at its own discretion.
Authors may also indicate up to three reviewers who should not be invited for assessment of the respective paper.

Back to top

Title Page

Please make sure your title page contains the following information.
Title
The title should be concise and informative.
Author information

- The name(s) of the author(s)
- The affiliation(s) of the author(s), i.e. institution, (department), city, (state), country
- A clear indication and an active e-mail address of the corresponding author
- If available, the 16-digit ORCID of the author(s)

If address information is provided with the affiliation(s) it will also be published.
For authors that are (temporarily) unaffiliated we will only capture their city and country of residence, not their e-mail address unless specifically requested.
Large Language Models (LLMs), such as ChatGPT, do not currently satisfy our authorship criteria. Notably an attribution of authorship carries with it accountability for the work, which cannot be effectively applied to LLMs. Use of an LLM should be properly documented in the Methods section (and if a Methods section is not available, in a suitable alternative part) of the manuscript.

Abstract

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.
For life science journals only (when applicable)

- Trial registration number and date of registration for prospectively registered trials

- Trial registration number and date of registration, followed by “retrospectively registered” for retrospectively registered trials

Statements and Declarations

The following statements should be included under the heading "Statements and Declarations" for inclusion in the published paper. Please note that submissions that do not include relevant declarations will be returned as incomplete.

- **Competing Interests:** Authors are required to disclose financial or non-financial interests that are directly or indirectly related to the work submitted for publication. Please refer to “Competing Interests and Funding” below for more information on how to complete this section.

Please see the relevant sections in the submission guidelines for further information as well as various examples of wording. Please revise/customize the sample statements according to your own needs.

Back to top

Text

Text Formatting

Manuscripts should be submitted in Word.

- Use a normal, plain font (e.g., 10-point Times Roman) for text.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use tab stops or other commands for indents, not the space bar.
- Use the table function, not spreadsheets, to make tables.
- Use the equation editor or MathType for equations.
- Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Manuscripts with mathematical content can also be submitted in LaTeX. We recommend using Springer Nature’s LaTeX template.

Headings

Please use no more than three levels of displayed headings.

Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

Footnotes

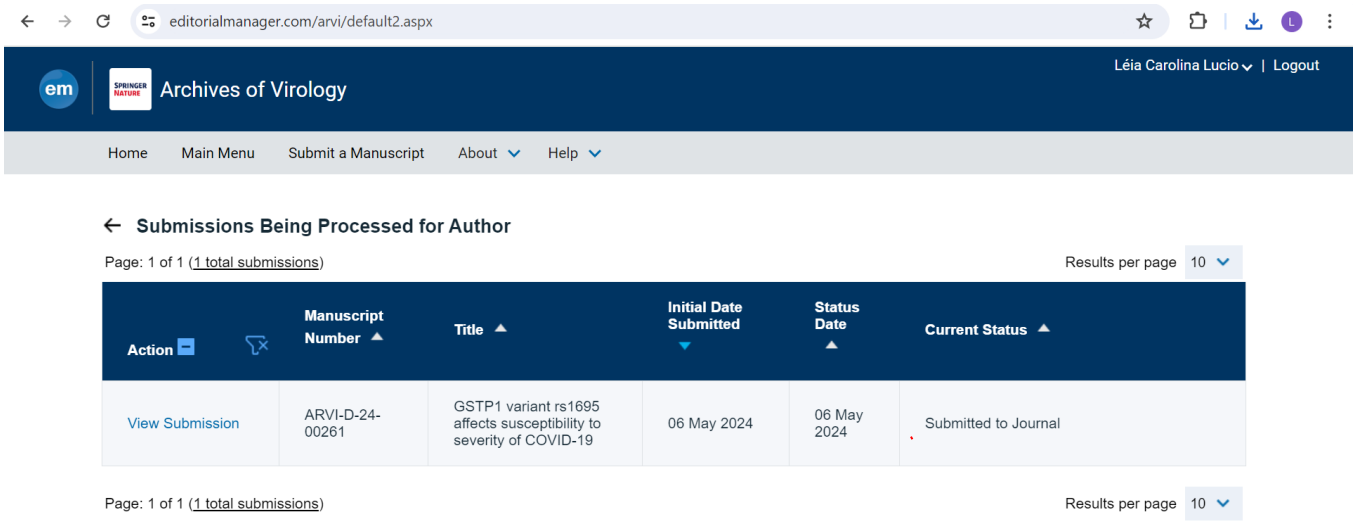
Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

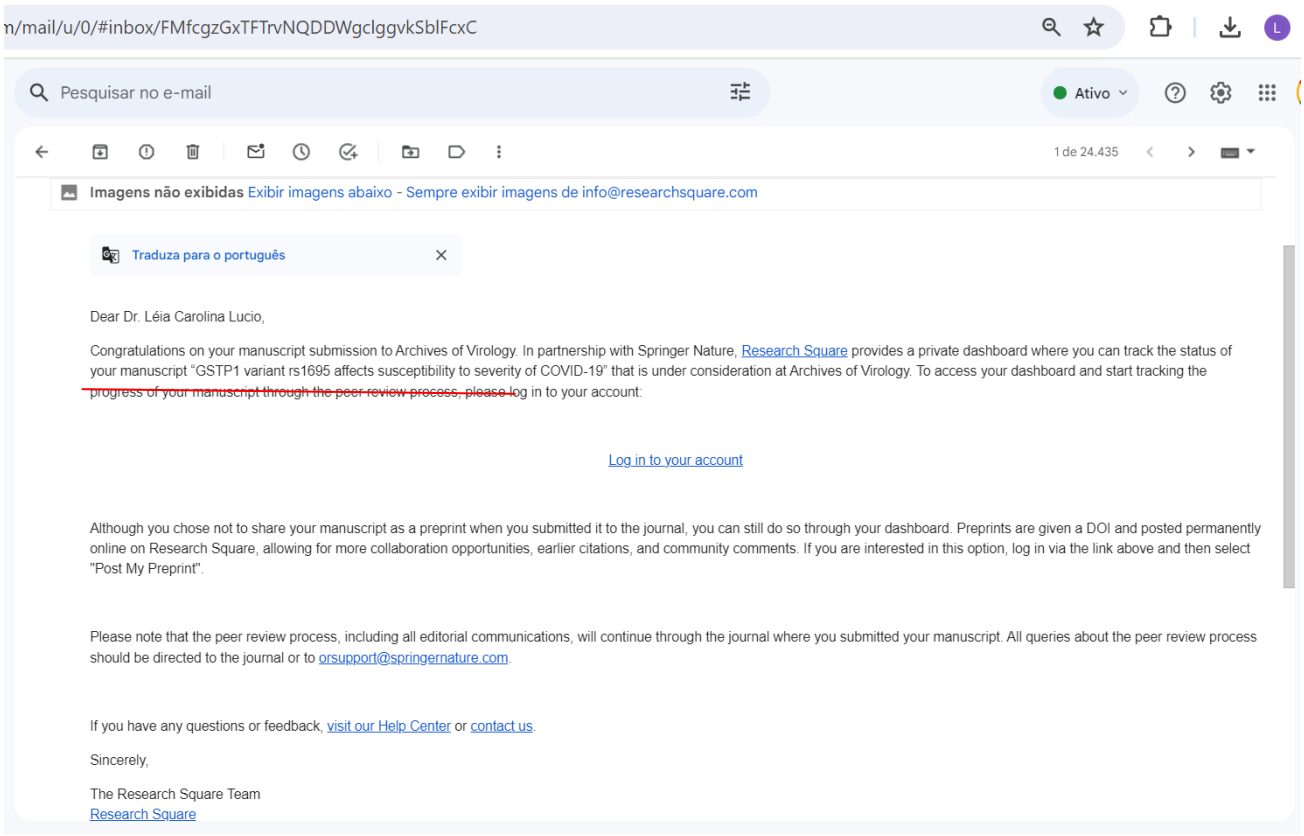
Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.
Always use footnotes instead of endnotes.

Acknowledgments

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

Comprovannte de Submissão ao periódico “Archives of Virology”




n/mail/u/0/#inbox/FMfcgzGxTFTrvNQDDWgclggvkSblFcxC
Pesquisar no e-mail
Ativo
1 de 24.435
Imagens não exibidas Exibir imagens abaixo - Sempre exibir imagens de info@researchsquare.com
Traduza para o português
Dear Dr. Léia Carolina Lucio,
Congratulations on your manuscript submission to Archives of Virology. In partnership with Springer Nature, Research Square provides a private dashboard where you can track the status of your manuscript "GSTP1 variant rs1695 affects susceptibility to severity of COVID-19" that is under consideration at Archives of Virology. To access your dashboard and start tracking the progress of your manuscript through the peer review process, please log in to your account:
Log in to your account
Although you chose not to share your manuscript as a preprint when you submitted it to the journal, you can still do so through your dashboard. Preprints are given a DOI and posted permanently online on Research Square, allowing for more collaboration opportunities, earlier citations, and community comments. If you are interested in this option, log in via the link above and then select "Post My Preprint".
Please note that the peer review process, including all editorial communications, will continue through the journal where you submitted your manuscript. All queries about the peer review process should be directed to the journal or to orsupport@springernature.com.
If you have any questions or feedback, visit our Help Center or contact us.
Sincerely,
The Research Square Team
Research Square