UNIVERSIDADE FEDERAL DE ALFENAS

DUILLIO ALVES CAIXETA

SOROPREVALÊNCIA DO *SARS-CoV-2* EM TRABALHADORES DE HOSPITAIS NA REGIÃO SUL DE MINAS GERAIS, BRASIL, NO ANO DE 2020

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Dissertação apresentada como parte dos requisitos para obtenção do título de Mestre em Ciências Biológicas pela Universidade Federal de Alfenas. Área de concentração: II - Interação Patógeno Hospedeiro.

Orientador: Prof. Dr. Luiz Cosme Cotta Malaquias
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A Banca examinadora abaixo-assinada aprova a Dissertação/Tese apresentada como parte dos requisitos para a obtenção do titulo de Mestra em Ciências Biológicas pela Universidade Federal de Alfenas. Área de concentração: Interação Patógeno-Hospedeiro.

Aprovada em: 10 de fevereiro de 2023.

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A toda pessoa, que de alguma forma, sofreu com o impacto da pandemia de Covid-19.

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RESUMO

A determinação da soroprevalência da infecção pelo SARS-CoV-2 pode ser realizada através da detecção de anticorpos anti-SARS-CoV-2 por meio de imunoensaios. Este levantamento é essencial para compreender, mediante estimativa, o desenvolvimento e a transmissão da doença na população estudada. Este estudo teve como objetivo realizar levantamento soroepidemiológico do SARS-CoV-2 em trabalhadores hospitalares em três cidades localizadas no sul do estado de Minas Gerais, Brasil, em 2020. Foram realizados testes imunoenzimáticos (ELISA) em soros de 859 participantes. A média de idade foi de 38 anos. Mulheres 71,4% e homens 28,6%; profissionais de saúde 74,5% e trabalhadores do setor administrativo 11,6%. Os sintomas relatados pelos participantes foram: febre 6,4%, dificuldade respiratória 5,8%, perda de olfato e paladar 7,0% e diarreia 15,8%. Relataram, ainda, contato com pacientes infectados 63,35% dos participantes. Apresentaram testes de ELISA positivos 21,7% dos indivíduos, 62,7% negativos e 15,6% indeterminados. O hospital 3 apresentou a maior positividade (22,9%), seguido pelo hospital 2 (21,6%) e pelo hospital 1 (20,3%) (p=0,079). Mulheres apresentaram maior prevalência que os homens (22,8% e 18,7% respectivamente). A maior positividade (22,0%) foi observada entre profissionais administrativos, seguida pelos profissionais de saúde (20,9%). Entretanto, os profissionais que realizavam exames laboratoriais e de imagem apresentaram maior positividade (30,3%), seguidos pelos administrativos (22,6%), área hospitalar Covid (22,0%) e hospitalar não-Covid (21,5%). Detectou-se correlação significativa entre os testes ELISA positivos e as variáveis: testes sorológicos pregressos, contato anterior com pacientes infectados e presença de febre, perda de olfato e paladar. Conclui-se que, os testes sorológicos foram, em período pré-vacinal, ferramentas importantes para a detecção de anticorpos contra o vírus entre os profissionais hospitalares, os testes ELISA demonstraram não haver diferença significativa entre os trabalhadores hospitalares das diversas áreas, entretanto demonstrou que os profissionais hospitalares de maneira geral estão expostos à infecção. Porém, apresentou-se, quando analisado os teses ELISA positivo, diferença significativa entre os sintomas relatados e contato com pacientes com Covid-19.

Palavras-chave: Covid-19; *SARS-CoV-2*; Soroepidemiologia; Hospitais; ELISA; Pandemia.

ABSTRACT

Determining the seroprevalence of SARS-CoV-2 infection can be performed by detecting anti-SARS-CoV-2 antibodies using immunoassays. The prevalence survey is essential to understand, through estimation, the development and transmission of the disease in the studied population. This study aimed to carry out a seroepidemiological survey of SARS-CoV-2 in hospital workers from three cities located in the south of the state of Minas Gerais, Brazil, in 2020. Enzyme-linked immunosorbent assays (ELISA) were performed on sera from 859 participants. The average age was 38 years. Women represented 71.4% and men 28.6%; health professionals were 74.5% and other workers in the administrative sector were 11.6%. The main symptoms reported by the participants were: fever 6.4%, difficulty breathing 5.8%, loss of smell and taste 7.0% and diarrhea 15.8%. 63.35% of the participants also reported contact with infected patients. 21.7% of the individuals presented positive ELISA tests, 62.7% were negative and 15.6% were undetermined. Hospital 3 had the highest positivity rate (22.9%), followed by hospital 2 (21.6%) and hospital 1 (20.3%) (p=0.079). Women had a higher prevalence positivity than men (22.8% and 18.7% respectively). The highest positivity in the test (22.0%) was observed among administrative professionals, followed by health professionals (20.9%). However, professionals who performed laboratory and imaging tests were more positive tests (30.3%), followed by administrative (22.6%), Covid hospital area (22.0%) and non-Covid hospital area (21.5%). A significant correlation was detected between positive ELISA tests and the following variables: previous serological tests, previous contact with infected patients, presence of fever, loss of smell and taste. It is concluded that the ELISA tests were, in the pre-vaccination period, important tools for the detection of antibodies against the virus among hospital professionals. The ELISA tests results showed no significant difference between hospital workers in different areas, nonetheless it demonstrated that hospital professionals in general are exposed to infection. Nevertheless, when analyzing the positive ELISA tests, there was a significant difference between reported symptoms and contact with patients with Covid-19.

Keywords: Covid-19; SARS-CoV-2; Seroepidemiology; Hospitals; ELISA; Pandemic.

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LISTA DE ABREVIATURAS E SIGLAS

ACE-2	Angiotensin Converting Enzyme 2 (Enzima Conversora de Angiotensina 2
SRAG	Síndrome Respiratória Aguda Grave
CLIA	*Chemiluminescent Immunoassay* (Imunoensaio Quimioluminescente)
EBOV	Ebola vírus
ELISA	*Enzyme-Linked Immunosorbent Assay* (Ensaio da Imunoabsorção Enzimática)
ERGIC	*ER-Golgi intermediate compartment* (Compartimento Intermediário do Complexo de Golgi)
H1N1	Hemaglutinina 1 Neuraminidase 1
H2N2	Hemaglutinina 2 Neuraminidase 2
H3N2	Hemaglutinina 3 Neuraminidase 2
HPI	Horas pós-infecção
IgA	Imunoglobulina A
IgG	Imunoglobulina G
IgM	Imunoglobulina M
LFIA	*Lateral Flow Immunoassay* (Imunoensaio de Fluxo Lateral)
MERS-CoV	*Middle East Respiratory Syndrome Coronavirus*
MG	Minas Gerais
mmHG	Milímetros de Mercúrio
mRNA	*Messenger Ribonucleic Acid* (Ácido Ribonucleico Mensageiro)
NSP	*Non-Structural Protein* (Proteína Não Estrutural)
OMS	Organização Mundial de Saúde
OPD	*o-Phenylenediamine Dihydrochloride* (Dicloridrato de o-Fenilenodiamina)
ORF	*Open Reading Frame* (Regiões Abertas de Leitura)
PaO_2FiO_2	*Partial pressure of arterial oxygen to the fraction of inspired oxygen* (Relação da pressão parcial de oxigênio arterial com a fração inspirada de oxigênio)
PBS	*Phosphate-buffered saline* (Tampão fosfato-salino)
PBS-T	*Phosphate-buffered saline - Tween* (Tampão fosfato-salino –

	Tween)
PP	Poliproteínas
RBD	*Receptor Binding Domain* (Domínio de Ligação do Receptor)
RNA	*Ribonucleic Acid* (Ácido ribonucleico)
RTC	*Replicase-Transcriptase Complex* (Complexo Replicase-Transcriptase)
RT-PCR	*Reverse Transcription Polymerase Chain Reaction* (Reação em cadeia da polimerase – transcriptase reversa)
SARS-CoV	*Severe Acute Respiratory Syndrome-Related Coronavirus*
SARS-CoV-2	*Severe Acute Respiratory Syndrome-Related Coronavirus 2*
sgRNA	*Single Guide Ribonicreic Acid* (RNA de guia único)
UFMG	Universidade Federal de Minas Gerais
UFRJ	Universidade Federal do Rio de Janeiro
UNIFAL-MG	Universidade Federal de Alfenas
USP	Universidade de São Paulo
UTI	Unidade de Tratamento Intensivo
UTR	*Untranslated Regions* (Regiões Não Traduzidas)
VOC	*Concern Variants* (Variantes de Preocupação)
VOC-LUM	*Variant of Concern Lineage Under Monitoring* (Linhagens de VOC sob monitoramento)
VOI	*Variants of Interest* (Variantes de Interesse)

SUMÁRIO

1 INTRODUÇÃO

O *SARS-CoV-2* é um vírus de RNA fita única que pertencente à família *Coronaviridae* e apresenta quatro proteínas estruturais, sendo a proteína spike (S), a responsável pela adsorção e penetração do vírus nas células hospedeiras (CHAN *et al.*, 2020). Essa proteína permite a ligação do vírus às células que possuem o receptor da enzima conversora de angiotensina-2 (ACE-2) (HOFFMANN *et al.*, 2020). O causador da Covid-19 é transmitido principalmente por meio de aerossóis liberados através das vias aéreas (HARRISON; LIN; WANG, 2020), o que torna a transmissão muito mais eficiente, pela sua rápida capacidade de disseminação (LEWIS, 2022).

Após a infecção, os infectados podem não apresentar sintomas (assintomáticos) ou apresentar diversas manifestações clínicas, que variam de sintomas gripais leves, moderados ou graves com o desenvolvimento da síndrome respiratória aguda grave (JIN *et al.*, 2020). Em março de 2020 foi declarada pandemia pela OMS, a partir daí foram registrados milhões de infecções e óbitos em todo o planeta (WHO, 2020a). Existem algumas técnicas para determinar a prevalência da infecção pelo *SARS-CoV-2* na população, dentre elas, a detecção de anticorpos anti-*SARS-CoV-2* por testes imunocromatrográficos ou ensaios imunoenzimáticos (ELISA) (HUERGO *et al.*, 2021).

O estudo da soroprevalência em período pré-vacinal é fundamental para compreender e quantificar o quanto daquela população teve contato com o vírus e qual grupo possui maior taxa de contágio (CAVATAIO; SCHNELL, 2021). Portanto, investigar como ocorreu a circulação da Covid-19 na região do Sul de Minas Gerais foi essencial para estabelecer estratégias e medidas de combate à doença em período pré-vacinal, visto que o levantamento soroepidemiológico auxiliou nas decisão e planejamento estratégico das políticas públicas de enfretamento à Covid-19.

Desta maneira, o presente trabalho teve como objetivo realizar um levantamento soroepidemiológico do *SARS-CoV-2*, em hospitais nas cidades de Alfenas, Varginha e Poços de Caldas, no Sul de Minas Gerais, Brasil, entre agosto e dezembro de 2020, através da quantificação de anticorpos IgG para proteína do nucleocapsídeo do *SARS-CoV-2* por meio de ensaios de ELISA, em período anterior ao início da vacinação, com grande índice de transmissão do vírus e com decretos estaduais e municipais de restrição de circulação das pessoas.

Nas seções seguintes serão apresentados revisão de literatura que descreve o

cenário da pandemia da Covid-19 durante a realização da pesquisa, cenário atual, caracterizando o vírus *SARS-CoV-2*, formas de transmissão, aspectos clínicos da doença, diagnóstico, aspectos epidemiológicos. Os resultados do trabalho foram incluídos na forma de artigo que foi submetido à revista *Brazilian Journal of Microbiology* (Anexo A).

2 REVISÃO DE LITERATURA

Os tópicos apresentados neste capítulo abordam os temas do trabalho que foram obtidos por meio do estudo de diversas publicações.

2.1 PANDEMIAS E EPIDEMIAS VIRAIS

A humanidade, desde os primórdios, foi acometida por doenças virais que causaram epidemias e pandemias infectando milhões de pessoas, assim como, milhões de óbitos (MORENS *et al.*, 2020). Embora os termos epidemia e pandemia tenham sido utilizados como sinônimos por um período, eles se diferem principalmente pelo tamanho da área geográfica em que acontecem. O termo pandemia é definido como uma doença causada por um agente biológico que possui rápida disseminação geográfica acontecendo globalmente, com alta taxa de infecciosidade, contagiosidade e gravidade, em sua grande maioria é um agente novo, ou uma nova variante de um organismo já existente, com baixa taxa de imunidade populacional. Já o termo epidemia é usado para caracterizar uma doença com as características acima, mas que se apresenta em uma área geográfica menor, como por exemplo em apenas um país (MORENS; FOLKERS; FAUCI, 2009).

O Dicionário de Epidemiologia define pandemia como sendo uma epidemia que acontece globalmente ou em área muito ampla, por sua vez, extrapola fronteiras internacionais e afeta um grande número de pessoas (MIQUEL PORTA, 2014). Entretanto, a OMS não apresenta uma definição específica para o termo, mas ao ser declarada a pandemia da Covid-19, o diretor geral usa o termo surto que pode ser caracterizado como uma pandemia, como uma forma de interesse público e de atenção em relação à gravidade da situação naquele momento (SINGER; THOMPSON; BONSALL, 2021; WHO, 2020b).

As pandemias e epidemias acontecem, com mais frequência, através de agentes infecciosos, como os vírus emergentes que se disseminam na população em uma determinada área geográfica depois do primeiro contato com os reservatórios, que podem ser os animais silvestres. Já os reemergentes são aqueles que já estão disseminados na população, mas que sofreram mutações (TROVATO *et al.*, 2020). Esses novos agentes infecciosos conseguem se disseminar com maior facilidade

porque o sistema imunológico ainda não o reconhece e não possui estratégias eficientes para sua eliminação, assim, causam inúmeras infecções e óbitos (NII-TREBI, 2017).

Dessa maneira, ao longo dos anos diversas pandemias e epidemias aconteceram com os seguintes vírus emergentes: a gripe espanhola causada pelo vírus *H1N1* em 1918, a gripe asiática causada pelo vírus *H2N2* em 1957, a gripe de Hong Kong causada pelo vírus *H3N2* em 1968, a Sars causada pelo vírus *SARS-CoV* em 2002, a gripe suína causada pelo vírus *H1N1pdm09* em 2009, a síndrome respiratória do Oriente Médio causada pelo vírus *MERS-CoV* em 2012, a ebola causada pelo vírus *EBOV* em 2013 e, recentemente, a pandemia da Covid-19 causada pelo vírus *SARS-CoV-2*. Todas elas causaram milhares de infeções e óbitos (AKIN; GÖZEL, 2020; JIN *et al.*, 2020; PEIRIS *et al.*, 2003).

2.2 BETACORONAVIRUS

Os Coronavírus pertencentes à família *Coronaviridae* e ao gênero *Betacoronavirus,* tem grande relevância epidemiológica, principalmente pelo momento pandêmico vivenciado. Dessa família, três vírus serão apresentados, o *Severe acute respiratory syndrome-related coronavirus* (*SARS-CoV*), o *Middle East respiratory syndrome-related coronavirus* (*MERS-CoV*) e *Severe acute respiratory syndrome-related coronavirus 2* (*SARS-CoV-2*) (ICTV, 2020).

O SARS-CoV causa a doença Sars, com sintomas semelhantes à gripe, com febre acima de 38ºC, tosse, dificuldade respiratória podendo evoluir para pneumonia e causar óbito (PEIRIS *et al.*, 2003; STADLER *et al.*, 2003). Tem como hospedeiro inicial os morcegos, infectando posteriormente civetas e humanos, este vírus causou em novembro de 2002, surto na província de Guangdong na China (CUI; LI; SHI, 2019) e até julho de 2003 se espalhou por 27 países, causando 8.096 infeções com 774 óbitos, sendo em julho de 2003 declarada fim da ameaça global (CHERRY, 2004; WIT *et al.*, 2016). No Brasil, foi registrado um caso em abril de 2003, porém foi retirado da lista posteriormente (CHERRY, 2004).

Dez anos após à Sars, outro coronavírus foi novamente encontrado em humanos, o *MERS-CoV*, este vírus foi idenditicado em junho de 2012 em um paciente de 60 anos em Jeddahm, na Arábia Saudita. Os pacientes infectados podem ser assintomáticos, ou desenvolver sintomas gripais e evoluir para a síndrome do

desconforto respiratório agudo, progredindo em diversos casos à óbito (CHAFEKAR; FIELDING, 2018). O reservatório deste vírus está relacionado aos camelos dromedários (WIT *et al.*, 2016). O *MERS-CoV* causou, até 2017, 2.040 casos com 712 óbitos em cerca de 27 países (CHAFEKAR; FIELDING, 2018). No continente americano foi encontrado apenas nos Estados Unidos (AZHAR *et al.*, 2019).

Outro vírus da família *Coronaviridae* é o *SARS-CoV-2* que causa a Covid-19, foi detectado pela primeira vez em Wuhan na China em dezembro de 2019 e em março de 2020 foi declarada pela OMS pandemia da Covid-19 (JIN *et al.*, 2020), causando milhões de infecções e óbitos em todo o mundo. Suas características estruturais, fisiológicas e epidemiológicas serão descritas nos tópicos seguintes.

2.3 SARS-COV-2

2.3.1 Origem do vírus *SARS-CoV-2*

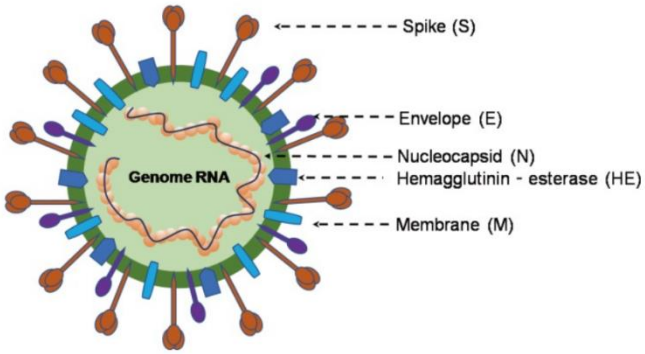
O *SARS-CoV-2* é um vírus zoonótico que tem sua origem provavelmente relacionada aos morcegos da espécie *Rhinolophus affinis* (LAM *et al.*, 2020) que são seus reservatórios naturais e por isso não lhes são patogênicos (RABI *et al.*, 2020). No entanto, estes não são os responsáveis pela transmissão aos humanos e o hospedeiro intermediário ainda é incerto (JO *et al.*, 2021).

Há evidências de que os pangolins malaios (*Manis javanica*) sejam esses hospedeiros, precisamente, o pangolin apresenta receptores de grande afinidade para o vírus, ao domínio de ligação do receptor (RBD) ao receptor da enzima conversora de angiotensina-2 (ACE-2), como acontece em humanos (RABI *et al.*, 2020). Em um trabalho analisando aminoácidos de RBD envolvidos na interação com ACE-2, mostrou que seis aminoácidos estão envolvidos nessa interação e são os mesmos encontrados nos CoVs dos pangolins malaios e diferem no pangolim chinês, confirmando a situação de possível hospedeiro intermediário (KADAM *et al.*, 2021). Estes animais não são encontrados naturalmente na China, mas são vendidos ilegalmente no país e são usados comumente na medicina popular chinesa e para alimentação (VOLPATO *et al.*, 2020).

2.3.2 Estrutura e ciclo de replicação do *SARS-CoV-2*

O *SARS-CoV-2* apresenta alta similaridade com genes codificadores de proteínas presentes nos demais coronavírus de morcegos, com exceção a proteína spike (S), ao serem comparados aos dois outros coronavírus que causaram surtos em humanos, o *SARS-CoV* apresentou similaridade genômica de 79% e o *MERS-CoV* de 50% (LU *et al.*, 2020). Seu genoma é de RNA de fita simples no sentido 5´-3´, possuindo como proteínas estruturais: Spike (S), Envelope (E), Membrana (M) e Nucleocapsídeo (N) (FIGURA 1), além de haver duas regiões não traduzidas (UTRs) e sete regiões abertas de leitura (ORF). Além dos genes estruturais, apresenta o gene replicase responsável pela codificação de 16 proteínas não estruturais que estão envolvidas no processo de replicação e transcrição (CHAN *et al.*, 2020; HU *et al.*, 2021).

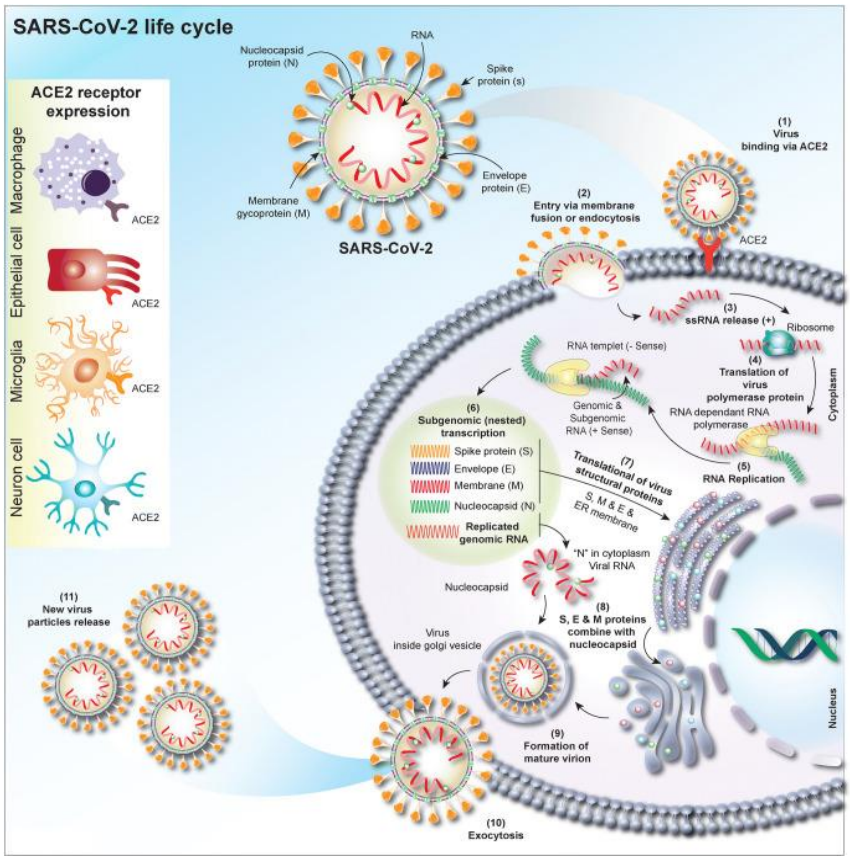
Figura 1 - Estrutura do *SARS-CoV-2.*



Fonte: (JIN *et al.*, 2020)

O *SARS-CoV-2* é disseminado através das vias aéreas, assim como sua infecção é por este mesmo meio (JIN *et al.*, 2020). Após a infecção, o vírus realiza os seguintes processos no seu ciclo de replicação: adsorção e penetração na célula; transcrição da replicase viral; transcrição e replicação genômica; tradução de proteínas estruturais, montagem e liberação de vírions (ARYA *et al.*, 2021; LU *et al.*, 2020; MACHHI *et al.*, 2020) (FIGURA 2).

Figura 2 - Ciclo de vida do *SARS-CoV-2.*



Fonte: (MACHHI *et al.*, 2020)

A adsorção e penetração na célula hospedeira acontece por meio da ligação entre a proteína S viral e o receptor da enzima conversora de angiotensina-2 (ACE-2) da célula hospedeira (HOFFMANN *et al.*, 2020).

Após a penetração, acontece a transcrição da replicase viral, em que, o RNA genômico (sgRNA) codifica duas ORFs a rep1a e a rep1b que expressam as poliproteínas pp1a e pp1ab (FEHR; PERLMAN, 2015; MACHHI *et al.*, 2020). Essas poliproteínas, pp1a e pp1ab, contêm os nsps 1–11 e 1–16, respectivamente, sendo posteriormente clivadas em nsps individuais (FEHR; PERLMAN, 2015). E, por fim, acontece o complexo replicase-transcriptase (RTC) através da reunião de nsps, que propiciam a síntese de RNA (FEHR; PERLMAN, 2015; MACHHI *et al.*, 2020). Na seguinte fase acontece a transcrição e replicação, no qual, o RNA genômico é utilizado como modelo para o processo de replicação que promove a sintetização do genoma (MACHHI *et al.*, 2020).

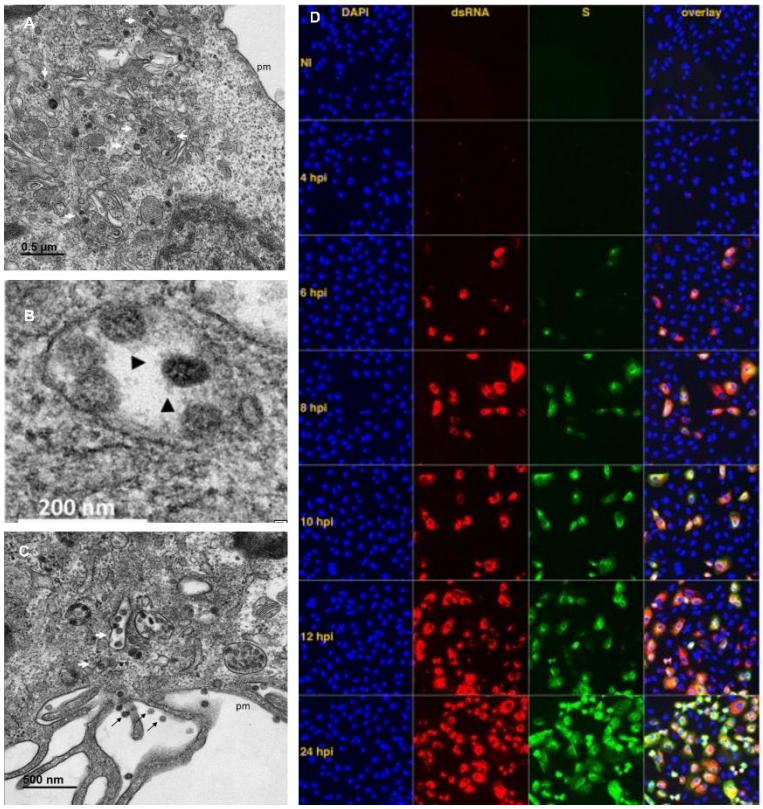
Com a transcrição finalizada, inicia a tradução dos ORF1a e ORF1b e além do RNA genômico, são produzidos nove outros RNAs subgenômicos (KIM *et al.*, 2020).

Acontece, também, a produção de proteínas estruturais transmembrana S, M e E, e acessórias que tem sua tradução no retículo endoplasmático, entretanto, a proteína N é traduzida nos ribossomos citosólicos (MACHHI *et al.*, 2020; MASTERS, 2006).

Finalmente acontece a montagem e liberação do vírion, estudos usando células Vero para analisar como é o processo de replicação viral, mostram que a montagem do vírion acontece em vesículas intracelulares, acredita-se que esteja relacionada ao aparelho de Golgi ou compartimento intermediário ER-Golgi (ERGIC) (FIGURA 3a), formando as estruturas pontiagudas do vírus e foi observada 8hpi em pequenas vesículas (FIGURA 3b) (EYMIEUX *et al.*, 2021). Sendo semelhante aos demais coronavírus e utilizam como mediadora a proteína M (KLUMPERMAN *et al.*, 1994; MACHHI *et al.*, 2020). Após serem analisadas dentro dessas vesículas, poucas horas depois foram observadas estruturas extracelulares que se moviam em direção à membrana celular, sugerindo a liberação por meio de exocitose do vírus (FIGURA 3c) (EYMIEUX *et al.*, 2021).

Através dos estudos realizados com células Vero, foi identificada periodicidade no ciclo de replicação do *SARS-CoV-2* em cerca de 8hpi, sendo a secreção viral detectada depois de 8 horas da infecção, com relativo aumento ao passar do tempo, houve também análise da quantificação do RNA viral intracelular que pode ser detectado 4hpi, aumentando até 10hpi com platô entre 10 e 12hpi (FIGURA 3d) (EYMIEUX *et al.*, 2021).

Figura 3 - Montagem e liberação do vírion de *SARS-CoV-2* em células Vero.



Fonte: (EYMIEUX *et al.*, 2021)

Legenda: a) A montagem do vírion acontece em vesículas intracelulares, como o aparelho de Golgi.
b) *SARS-CoV-2* observado 8hpi em pequenas vesículas.
c) Movimentação do vírus à membrana celular, sugerindo a liberação por meio de exocitose.
d) Secreção viral e mRNA detectados depois de 4, 6, 8, 10, 12, 24 hpi.

2.4 DOENÇA COVID-19

2.4.1 Formas de transmissão

O vírus *SARS-CoV-2* pode ser transmitido de três formas básicas: por meio de aerossóis, gotículas de saliva, fômites e fecal-oral (DHAMA *et al.*, 2020; HARRISON; LIN; WANG, 2020). A forma aerossol/gotículas de saliva acontece de humano para humano, em que, os aerossóis liberados ao falar, tossir ou espirrar são liberadas contendo o vírus, podendo o paciente ser sintomático ou assintomático e o vírion tem a capacidade de colonizar e replicar na garganta no início da infecção. Por isso é

necessário o uso de medidas para se evitar a transmissão, como o uso de máscaras, rastreamento dos contatos e isolamento físico (DHAMA *et al.*, 2020; GAO *et al.*, 2021; HARRISON; LIN; WANG, 2020; JIN *et al.*, 2020; TO *et al.*, 2021).

Entretanto, existe uma diferença entre a transmissão por meio de gotículas de saliva (*droplets*) e aerossóis, o que torna os aerossóis muito mais transmissíveis. Os *droplets* são partículas com cerca de mais de 5 μm e aerossóis com menos que 5 μm de diâmetro. Com isso, os aerossóis são estruturas que podem ser disseminados com maior agilidade e serem inalados com maior facilidade causando infecções alveolares. Na forma de aerossóis, o vírus permanece viável por cerca de 3 horas (JAYAWEERA *et al.*, 2020).

Os fômites são materiais inanimados que podem ser transmissores da doença, por meio de transmissão passiva, porque neles houve instalação do vírus que foi liberado pelos aerossóis, embora apresente menor taxa de transmissão (HARRISON; LIN; WANG, 2020). Em algumas superfícies eles podem permanecer ativo por diversas horas, como por exemplo: no aço inoxidável e plástico a permanência é de 72 horas, no papelão de 24 horas, no cobre de 04 horas e em aerossolizados e poeira entre 40 minutos e 30 minutos (DOREMALEN *et al.*, 2020). Portanto, materiais que podem ser considerados fômites são as maçanetas, corrimões, superfícies compartilhadas em supermercados, caixas eletrônicos, maquininhas de cartão, dinheiro, dentre outros.

A transmissão fecal-oral é caracterizada pela presença do vírus em materiais fecais que vieram a ter contato com a cavidade oral (HARRISON; LIN; WANG, 2020), dessa maneira, diversos estudos relatam a presença do *SARS-CoV-2* nas fezes e urina (JONES *et al.*, 2020), em um estudo realizado com pacientes positivos para a doença, cerca de 29% das amostras fecais tiveram resultado positivo para o vírus, porém, nenhuma amostra encontrou o vírus na urina (WANG *et al.*, 2020).

Em outro estudo, foram coletadas amostras em fezes de 28 pacientes positivos para a doença, sendo desses, 12 com RNA positivo nas amostras, o que sugere a possibilidade da transmissão fecal-oral, como também fecal-respiratórias por meio de fezes aerossolizadas (XIAO *et al.*, 2020). Assim como, quando realizado um estudo observando a infecção por *SARS-CoV-2* em enterócitos em 24hpi analisou-se a presença rara do vírus, no entanto, eles se espalharam por todo o organoide após 60hpi, isso acontece porque há existência de ACE-2 nessas células (LAMERS *et al.*, 2020).

Já em relação a urina, a carga viral encontrada foi baixa, o vírus identificado 12 dias pós-infecção, mas não foi possível determinar a infecção dos órgãos do sistema urinário, entretanto, sugere-se que possa ocorrer a infecção fecal/urinaria-respiratória (SUN *et al.*, 2020).

2.4.2 Manifestações clínicas

A doença Covid-19 pode ser caracterizada por quatro tipos básicos de manifestações clínicas: assintomáticos, sintomas leves, moderados e graves desenvolvendo a síndrome respiratória aguda grave (GAO *et al.*, 2021; JIN *et al.*, 2020). Os casos assintomáticos são de grande relevância epidemiológica, visto que, mesmo que não apresentem sintomas são potenciais transmissores do vírus (CHILAMAKURI; AGARWAL, 2021; GAO *et al.*, 2021; JIN *et al.*, 2020; TO *et al.*, 2021). Acredita-se que cerca de 50% das infecções são causadas por meio do contato com pessoas assintomáticas (ORTIZ-PRADO *et al.*, 2020).

As pessoas que desenvolvem sintomas leves podem apresentar febre, fadiga, tosse, anorexia, mal-estar, dores musculares, dor de garganta, dispneia, congestão nasal, perda de olfato e paladar, mas não apresentam alterações em imagens do tórax (CHILAMAKURI; AGARWAL, 2021; GAO *et al.*, 2021; HOZHABRI *et al.*, 2020; MACHHI *et al.*, 2020). Além de sintomas gastrointestinais como náuseas, vômitos, perda de apetite, diarreia e dor abdominal (JONES *et al.*, 2020).

Sintoma como febre é mais comum e desenvolvem em 80% dos pacientes. Por sua vez, tosse entre 50% e 80% e dispneia cerca de 30% (CHEN *et al.*, 2020; HARRISON; LIN; WANG, 2020; ORTIZ-PRADO *et al.*, 2020). Estes sintomas leves podem cessar após a primeira semana de infecção, entretanto, podem ser persistentes ou evoluírem a estágios mais graves (MACHHI *et al.*, 2020) e iniciarem entre 2 e 14 dias após a exposição ao vírus (CDC, 2021). Assim, pessoas sintomáticas são consideradas mais contagiosas (HOZHABRI *et al.*, 2020). Por sua vez, quando analisado os sintomas levando em consideração à variante Ômicron, o paciente apresenta dor de garganta (45,3%), fadiga/dor nas articulações (42,6%) e tosse (33,1%) como sintomas mais comuns (KIRCA *et al.*, 2022).

Os sintomas moderados apresentam manifestações clínicas mais intensas e em imagem do tórax com manifestação leve de pneumonia (GAO *et al.*, 2021). Tosse seca, taquipneia e falta de ar são os sintomas mais comuns (HOZHABRI *et al.*, 2020).

Esses pacientes, assim como os casos leves, são maioria como relatado em um estudo com mais de 72.314 análises de Covid-19 na China, desde o início do surto até 11 de fevereiro de 2020, e apresentou cerca de 81% dos casos, os pacientes se recuperaram em até 10 dias após a infecção (WU; MCGOOGAN, 2020).

Os pacientes que desenvolvem os sintomas graves e críticos apresentam falta de ar com frequência respiratória $\geqslant$ 30 respirações/minuto, saturação de oxigênio $\leqslant$ 93%, PaO_2/FiO_2 (relação da pressão parcial de oxigênio arterial com a fração inspirada de oxigênio) $\leqslant$ 300 mmHg, com imagem do tórax apresentando lesões com progressão de 50% entre 24–48h, acontecendo progressão rápida da doença com insuficiência respiratória, necessidade de ventilação mecânica, além de falência dos órgãos e necessidade de tratamento em UTI (GAO *et al.*, 2021). Dessa maneira, a síndrome respiratória aguda grave (SRAG) é a principal responsável pela mortalidade em doenças respiratórias (JIN *et al.*, 2020).

Os graves danos aos pulmões são causados pela resposta imune contra o *SARS-CoV-2*, em que são secretadas citocinas inflamatórias e preparam as células T e B para a resposta imunológica, no entanto, o excesso dessa secreção causada pela disfunção da resposta imune, provocada pelo vírus causa graves danos aos pulmões e pode evoluir à inflamação sistêmica (CHUNG; THONE; KWON, 2021; VABRET *et al.*, 2020).

Além do sistema respiratório, diversos sistemas podem ser afetados pela Covid-19 como o nervoso, cardiovascular, excretor, gastrointestinal e em aspectos neurológicos causando diversas manifestações clínicas que aumentam a virulência (MACHHI *et al.*, 2020). E a doença pode ser agravada quando os pacientes apresentam comorbidades como doenças cardiovasculares, renais, disfunção hepática, diabetes, doença de Parkinson e câncer (CHILAMAKURI; AGARWAL, 2021).

De acordo com os resultados de uma revisão sistemática de meta-análise, que estudou 61 trabalhos relacionados aos fatores de risco relacionados às comorbidades e a Covid-19, além das já citadas acrescenta doenças digestivas, respiratórias e pessoas fumantes (FANG *et al.*, 2020). As doenças: renal, cerebrovascular, do sistema respiratório, cardiovascular, hipertensão, pulmonar obstrutiva crônica e diabetes foram associadas a pacientes que vieram a óbito, respectivamente em grau de ocorrência (FANG *et al.*, 2020).

O estudo também apontou que o sexo masculino é mais propenso a desenvolver doença grave e óbito (FANG *et al.*, 2020). Outro fator de risco para a doença é a idade, pessoas com idades avançadas estão mais susceptíveis a desenvolver a doença grave e óbito, isso acontece porque essas pessoas possuem maior fragilidade do sistema imune (FANG *et al.*, 2020; HARRISON; LIN; WANG, 2020).

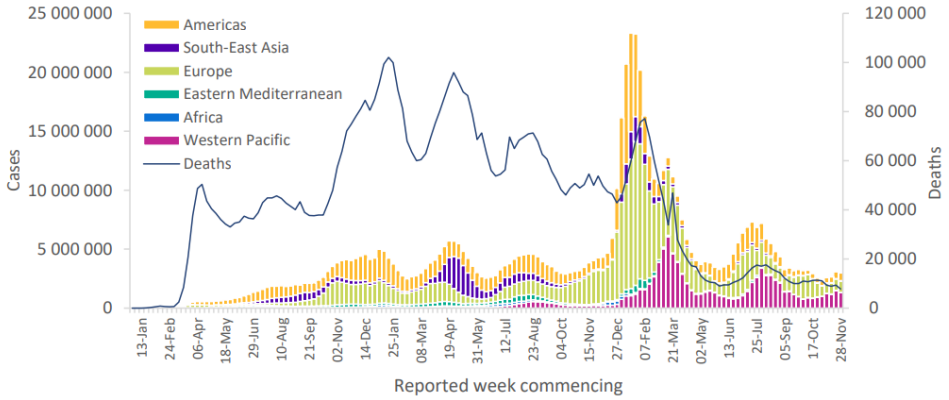
2.5 PANDEMIA DA COVID-19

O vírus *SARS-CoV-2* foi identificado pela primeira vez na cidade de Wuhan, Província de Hubei na China, em 12 de dezembro de 2019. Foi inicialmente chamado de 2019-nCoV e em fevereiro de 2020 a OMS nomeou a doença causada por esse agente etiológico de Doença do Coronavírus 2019 ou Covid-19 (DHAMA *et al.*, 2020). Em 14 de janeiro de 2020 foi confirmada a transmissão humano-humano em membros da mesma família (WHO, 2020c) e em 11 de março de 2020 foi declarada pandemia da Covid-19 pela OMS (WHO, 2020a).

O vírus se espalhou rapidamente pelo planeta e em 31 de março de 2020, final do mês em que a pandemia foi declarada, já haviam sido confirmados globalmente 750.890 casos e 36.405 óbitos (WHO, 2020a). Após seis meses da declaração da pandemia, em 28 de setembro de 2020, 32.730.945 milhões de casos e 991.000 óbitos (WHO, 2020e). Um ano de declaração da pandemia, em 30 março de 2021 o boletim epidemiológico da OMS apresentava globalmente 126.372.442 de casos e 2.769.696 óbitos, desses, 3,8 milhões de casos em uma semana com 640.000 óbitos (WHO, 2021).

Mais de dois anos de pandemia, em 5 de junho de 2022, apresentam-se 529.688.157 casos confirmados e 6.297.872 óbitos (WHO, 2022a). Atualmente, em 7 de dezembro de 2022, apresentam-se 641.487.094 casos confirmados e 6.621.419 óbitos (WHO, 2022b). A Figura 4 apresenta um gráfico da evolução de casos e óbitos globalmente, e pode-se observar a evolução do número de confirmações e óbitos por região do planeta desde o início das contaminações, em dezembro de 2019, até o mês de dezembro de 2022 (WHO, 2020d).

Figura 4 - Casos e óbitos de Covid-19 relatados semanalmente pela OMS por regiões globais.

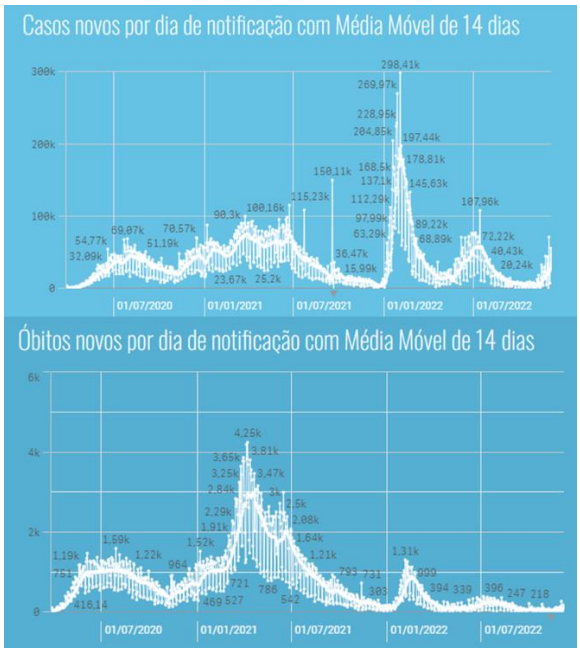


Fonte: (WHO, 2020d)

No contexto nacional, em 31 de março de 2020 o número acumulado de casos confirmados no país foi de 5.744 casos e 201 óbitos. Após seis meses da declaração da pandemia, em 28 de setembro de 2020, foram 4.745.464 casos e 142.058 óbitos. Em 30 março de 2021 foram de 12.658.109 casos e 317.646 óbitos. E em 21 de setembro de 2021 foram 21.381.790 e 595.446 óbitos (BRASIL, 2022b).

Em 5 de junho de 2022, o número de casos acumulados foi de 31.159.335 casos e 667.005 óbitos (BRASIL, 2022b). Atualmente, em 8 de dezembro de 2022 apresentam-se 35.531.716 casos e 690.677 óbitos (BRASIL, 2022b). Na figura 5 pode-se observar a evolução de casos confirmados e óbitos no Brasil de março de 2020 a dezembro de 2022.

Figura 5 - Casos novos de Covid-19 por dia de notificação no Brasil.

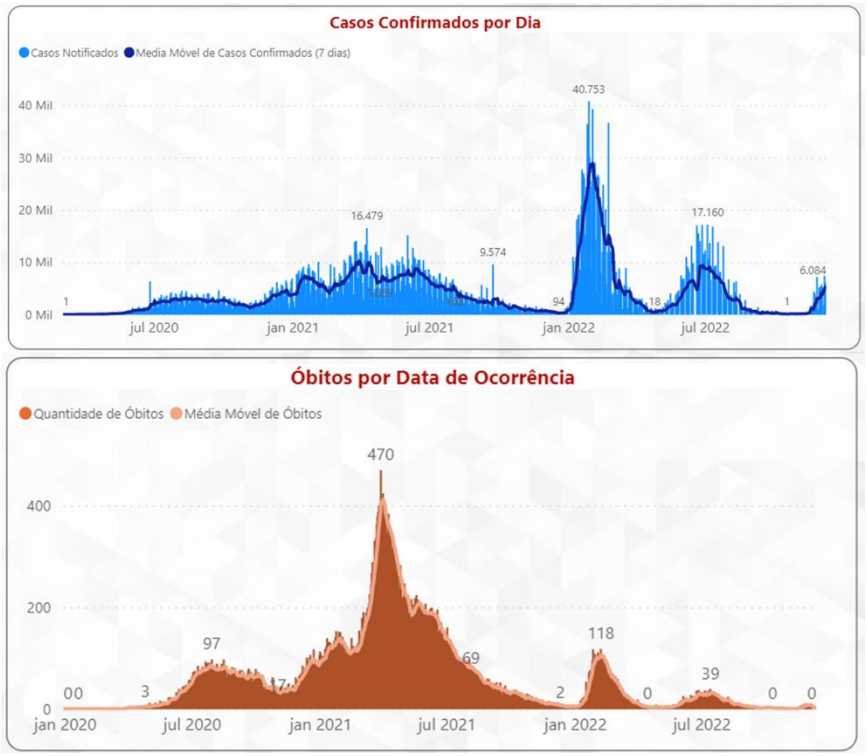


Fonte: (BRASIL, 2022b)

No Estado de Minas Gerais, da mesma forma demonstrado acima nos aspectos global e nacional, em 31 de março de 2020 já haviam sido confirmados 275 casos e 2 óbitos (MINAS GERAIS, 2020a). Após seis meses da declaração da pandemia, em 28 de setembro de 2020, foram 290.137 casos e 7.240 óbitos (MINAS GERAIS, 2020b). Em 30 março de 2021 foram 111.893 casos e 23.915 óbitos (MINAS GERAIS, 2021a). E em 21 de setembro de 2021 foram 2.114.292 casos e 54.095 óbitos (MINAS GERAIS, 2021b).

Em 6 de junho de 2022, o número de casos acumulados foi de 3.446.478 casos e 61.579 óbitos (MINAS GERAIS, 2022a). Atualmente, em 07 de dezembro de 2022 apresentam-se 3.952.942 casos e 64.038 óbitos (MINAS GERAIS, 2022b). Na Figura 6 pode-se observar a evolução de casos confirmados e óbitos em Minas Gerais de março de 2020 a dezembro de 2022.

Figura 6 - Casos novos de Covid-19 por dia de notificação em Minas Gerais.



Fonte: (MINAS GERAIS, 2022c)

2.5.1 Variantes

Com a alta taxa de infecção do vírus acontece, consequentemente, a ocorrência de mutações, com isso há o surgimento de novas variantes, em relação ao *SARS-CoV-2*, as mutações que chamaram maior atenção são as relacionadas à região RBD da proteína S (BIAN *et al.*, 2021). Existem atualmente diversas variantes monitoradas pelas agências de saúde e serão apresentadas a seguir (WHO, 2022c).

A Organização Mundial de Saúde define as variantes em dois grupos: as variantes de preocupação (VOC) e as variantes de interesse (VOI). As VOIs são aquelas que possuem modificações genéticas que alteram as características do vírus, podendo aumentar sua transmissibilidade, a virulência, reduzir a efetividade das vacinas, diagnósticos e tratamentos; já as VOCs são aquelas que apresentam as características acima, porém com aumento da transmissibilidade, da virulência e mudança no estado clínico da doença, além de poderem afetar a eficácia das vacinas, terapias e diagnósticos (WHO, 2022c).

As VOI não estão em circulação atualmente e são oito: a Épsilon encontrada

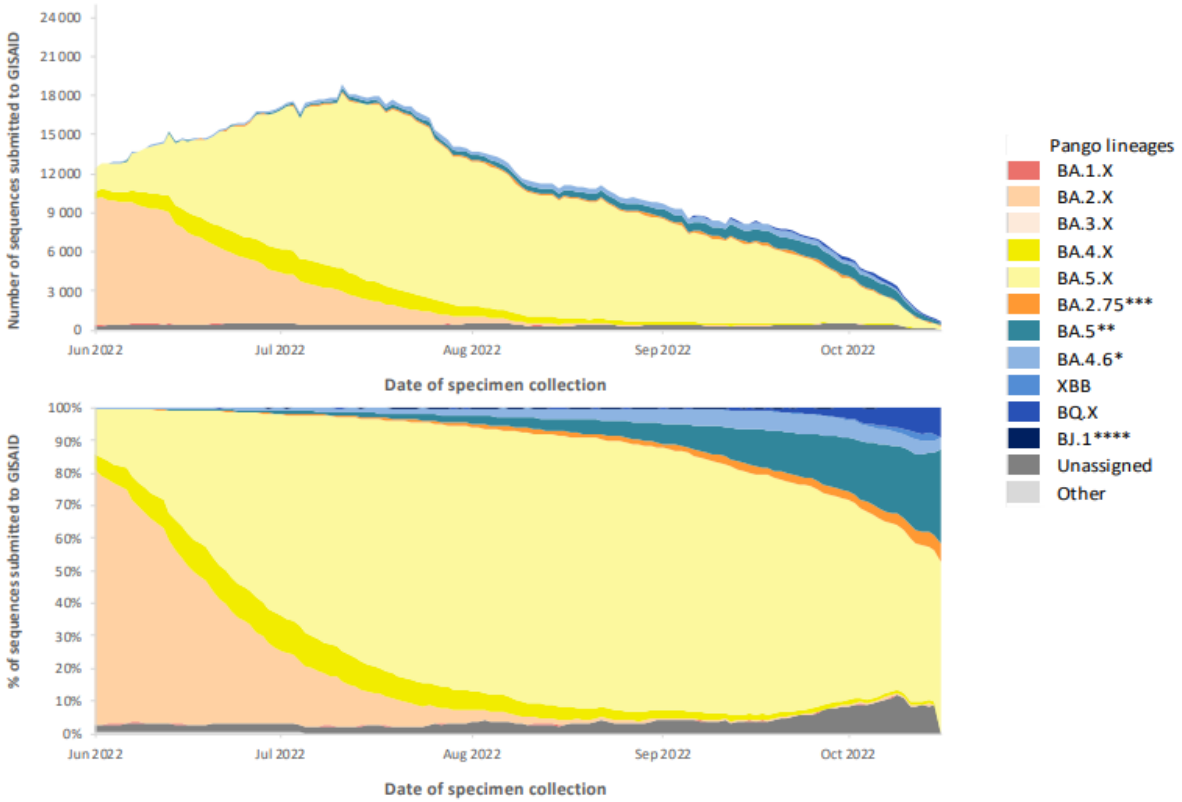
nos Estados Unidos em março de 2020, a Zeta encontrada no Brasil em abril de 2020, a Eta encontrada em vários países em dezembro de 2020, a Theta encontrada nas Filipinas janeiro de 2020, a Kappa encontrada na Índia em outubro de 2020, a Lambda encontrada no Peru em dezembro de 2020; e a Mu encontrada na Colômbia em janeiro de 2021 (WHO, 2022c).

As VOCs que circularam anteriormente foram quatro: a Alpha encontrada no Reino Unido em setembro de 2020; a Beta encontrada na África do Sul em maio de 2020; a Gamma encontrada no Brasil em novembro de 2020; e a Delta encontrada na Índia em outubro de 2020. Atualmente, a VOC em disseminação é a Ômicron encontrada em vários países em novembro de 2021 (WHO, 2022c).

Visto a importância epidemiológica da variante Ômicron, por sua transmissão acontecer globalmente, a OMS criou uma classificação particular para suas linhagens, chamadas de Linhagens de VOC sob monitoramento ou VOC-LUM (WHO, 2022c). São elas: BA.4 e BA.5 encontradas na África do Sul em janeiro de 2021, BA.2.12.1 encontrada nos Estados Unidos em dezembro de 2021, BA.2.9.1 e BA.2.13 encontradas em vários países em fevereiro de 2022, BA.2.11 encontrada em vários países em março de 2022 (WHO, 2022c). Com o alto índice de infecção, além das linhagem conhecidas da variante Ômicron, surgiram diversas sublinhagens que apresentam grande importância epidemiológica, como a BQ.1* que é uma sublinhagem de BA.5 (WHO, 2022g).

Na Figura 7, pode-se analisar o número e a porcentagem de sequências de RNA de *SARS-CoV-2* globalmente de junho a 24 de outubro de 2022. As linhagens da variante Ômicron são as mais encontradas, o que reafirma a importância do monitoramento dessa variante e de suas linhagens (WHO, 2022d).

Figura 7 - O número e a porcentagem de sequências de SARS-CoV-2.

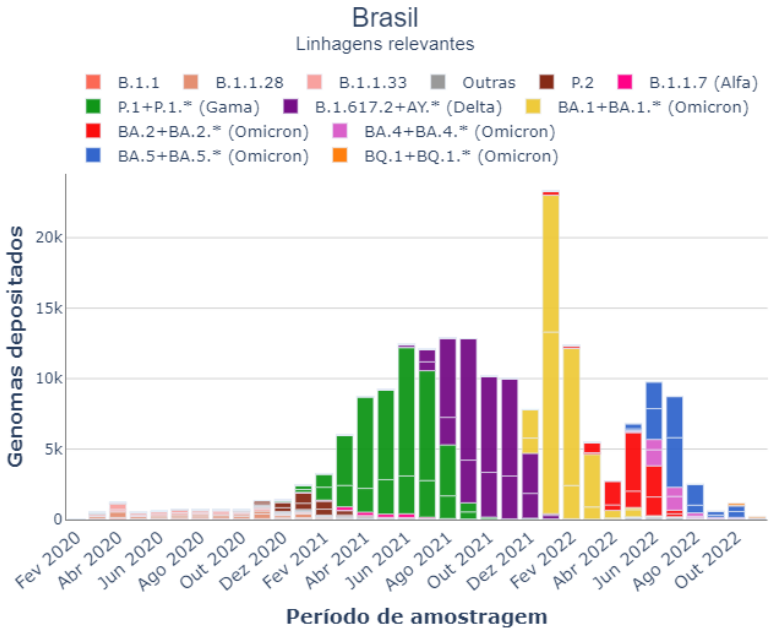


Fonte: (WHO, 2022d)

Legenda: O gráfico 1 mostra o número e o 2 a porcentagem de todas as variantes circulantes desde junho de 2022. Linhagens irmãs Ômicron e adicionais As linhagens descendentes de Ômicron VOC sob monitoramento adicional são mostradas. BA.1.X, BA.2.X, BA.3.X, BA.4.X e BA.5.X incluem todos os BA.1, BA.2, BA.3, BA.4 e linhagens descendentes combinadas BA.5, exceto as subvariantes Ômicron sob monitoramento mostradas individualmente. A categoria Não atribuído inclui linhagens pendentes para um nome de linhagem Pango, enquanto a categoria Outros inclui linhagens que são atribuídas, mas não listadas na legenda. Fonte: Dados da sequência do SARSCoV-2 e metadados do GISAID, em 24 de outubro de 2022.

A Figura 8 mostra quais variantes estavam em circulação desde fevereiro de 2020 até novembro de 2022 no Brasil, observando que a partir de janeiro de 2022 a variante Ômicron foi a mais observada.

Figura 8 – Variantes em circulação no Brasil entre fevereiro de 2020 e novembro de 2022.



Fonte: (FIOCRUZ, 2022)

2.5.2 Vacinas

As vacinas são estratégias fundamentais para reduzir a circulação de doenças, visto que as respostas imunológicas humorais estão relacionadas aos anticorpos neutralizantes (SHIH *et al.*, 2020). Existem atualmente diversas tecnologias que são empregadas para a produção de vacinas da Covid-19 baseadas em: vírus atenuados *SARS-CoV-2*, vírus *SARS-CoV-2* inativados, proteínas *SARS-CoV-2*, DNA, mRNA vetores virais (FORNI *et al.*, 2021).

De acordo com a OMS, até 06 de dezembro de 2022 existiam 199 candidatas a vacina na fase pré-clínica e 175 vacinas candidatas na fase clínica, dessas, 53 encontram-se na fase 1, 30 na fase 1/2, 14 na fase 2, 16 na fase 2/3, 49 na fase 3 e 11 na fase 4 (WHO, 2022f). Ainda de acordo com a OMS, até 08 de novembro de 2022, 44 vacinas entraram com processo de análise e aprovação para aplicação na população, diversas ainda aguardam aprovação e outras já se encontram com o processo finalizado (WHO, 2022e).

Dessa maneira, até 5 de dezembro de 2022, foram aplicadas globalmente

12.998.974.878 doses de vacinas, sendo 5.545.594.946 pessoas vacinadas com pelo menos uma dose e 5.004.887.244 com esquema vacinal completo (WHO, 2022b).

Em relação ao Brasil, o início da Campanha Nacional de Vacinação contra a Covid-19 foi em 18 de janeiro de 2021 e até dezembro de 2022, as vacinas aprovadas e distribuídas para uso são: Vacina adsorvida Covid-19 (inativada) Sinovac/Instituto Butantan, Vacina Covid-19 (vetor viral) AstraZeneca/Fiocruz, Vacina Covid-19 (mRNA) (Comirnaty) Pfizer/Wyeth e Vacina Covid-19 (vetor viral) Janssen (BRASIL, 2022a).

A vacina adsorvida Covid-19 (inativada) Sinovac/Instituto Butantan utiliza a tecnologia do antígeno inativado do *SARS-CoV-2*, nos testes realizados com intervalo de 28 dias entre a primeira e segunda dose apresentou soroconversão de 97% e sua eficácia para prevenção de casos sintomáticos que necessitaram de assistência médica foi de 77,96%. Ela pode ser aplicada em pessoas acima de 6 anos (BRASIL, 2022a).

A vacina Covid-19 (vetor viral) AstraZeneca/Fiocruz contém partículas virais do adenovírus recombinante de chimpanzés não replicante, mas expressa a glicoproteína S do *SARS-CoV-2*, nos testes de soroconversão, ela apresentou ≥ 98% 28 dias depois da primeira dose e > 99% no mesmo período após a segunda dose. Sua eficácia é de 73,43% tanto em pessoas com comorbidades quanto na população em geral. Entretanto, esta vacina não está autorizada para uso em indivíduos menores de 18 anos (BRASIL, 2022a).

A vacina Covid-19 (mRNA – encapsulação) (Comirnaty) Pfizer/Wyeth contém o RNAm codificante da proteína S do *SARS-CoV-2*[1], apresenta eficácia de 95,5% 7 dias após a segunda dose. Ela pode ser aplicada a partir de 5 anos de idade, sendo de 5 a 11 anos e 11 meses administrada a dose infantil (BRASIL, 2022a).

E a vacina Covid-19 (vetor viral) Janssen contém adenovírus do tipo 26 e codifica a glicoproteína S do *SARS-CoV-2*, foi inicialmente indicada para aplicação em dose única e apresenta cerca de 66,3% de eficácia após 14 dias de vacinação, havendo variações nesse valor em determinadas regiões geográficas pelo fato da infecção por diferentes variantes do vírus. Ela não é indicada para pessoas menores de 18 anos (BRASIL, 2022a).

No entanto, observando a fragilidade imunológica de alguns grupos específicos, iniciou-se a partir de 15 de setembro de 2021 a administração da dose de

[1] O mRNA é posteriormente revestido em uma camada de nanopartícula lipídica complexa.

reforço para pessoas acima de 70 anos e pacientes com alto grau de imunossupressão, e a partir de 28 de setembro de 2021, estendeu-se para a população acima de 60 anos, assim como para os profissionais de saúde a partir de 6 meses após finalização do esquema vacinal primário. A partir de 17 de novembro do mesmo ano, a dose de reforço foi ampliada para todas as pessoas acima de 18 anos, visto a redução da efetividade das vacinas com o passar do tempo (BRASIL, 2022a).

Entretanto, com a rápida disseminação da variante Ômicron, recomendou-se a dose de reforço para 4 meses após completar o esquema vacinal (BRASIL, 2022a). A partir de 23 de abril de 2022 indicou-se uma quarta dose com intervalo de 4 meses após a dose de reforço para pessoas acima de 80 anos, essa dose adicional foi ampliada a partir de 6 de maio de 2022 para pessoas acima de 60 anos (BRASIL, 2022a).

Na tabela 1 pode-se observar de maneira sistematizada a farmacêutica, tecnologia da vacina, faixa etária de aplicação e intervalo entre as doses.

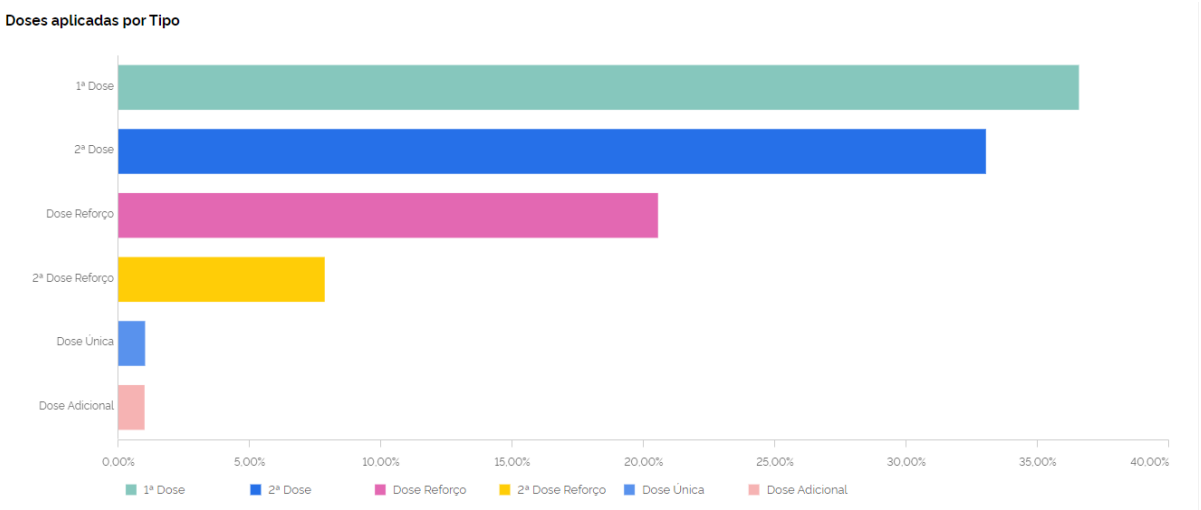
Tabela 1 - Vacinas aplicadas no Brasil contra a Covid-19.

Farmacêutica	Sinovac/ Instituto Butantan	AstraZeneca/ Fiocruz	Pfizer/ Wyeth	Pfizer/ Wyeth	Janssen
Tecnologia da vacina	vacina adsorvida Covid-19 (inativada)	vacina Covid-19 (vetor viral)	vacina Covid-19 (RNAm)	vacina Covid-19 (RNAm)	Vacina Covid-19 (vetor viral)
Faixa etária	a partir de 6 anos	a partir de 18 anos	a partir de 12 anos	entre 5 anos e 11 anos e 11meses	a partir de 18 anos
Intervalo entre as doses (esquema primário)	4 semanas	4 a 8 semanas	3 a 12 semanas	8 semanas	dose única

Fonte: (BRASIL, 2022a)

Desde o início do plano de imunização no país, foram aplicadas até 10 de dezembro de 2022 495.435.670 doses de vacinas para prevenção da Covid-19, sendo, 181.169.932 (36,57%) primeira dose, 163.622.663 (33,03%) segunda dose, 5.029.951 (1,02%) dose única, 101.777.062 (20,54%) dose de reforço, 38.908.953 (7,85%) segunda dose de reforço e 4.927.109 (0,99%) dose adicional (FIGURA 9) (BRASIL, 2022c).

Figura 9 – Porcentagem de doses de vacina contra a Covid-19 aplicadas no Brasil.



Fonte: (BRASIL, 2022d)

Como exposto acima, visto a alta transmissão da variante Ômicron, ela e suas linhagens são um dos fatores de preocupação em relação a efetividade da vacinação, porque é a variante mais mutada atualmente, que propicia o aumento de sua transmissibilidade e resistência parcial à imunidade adquirida pela vacinação, podendo levar a novos surtos da doença (ARAF *et al.*, 2022). Isso acontece porque a variante Ômicron apresenta cerca de 30 mutações na proteína S (CALLAWAY, 2021; KARIM; KARIM, 2021), proteína responsável pela entrada do vírus nas células do hospedeiro (CHAN *et al.*, 2020). Confirmando a problemática com a vacinação, essa variante foi identificada em pessoas já vacinadas (ARAF *et al.*, 2022).

2.6 SOROEPIDEMIOLOGIA

Em período vacinal e pós-vacinal, o estudo sorológico pode perder sua função como determinante de anticorpos contra a infecção pelo vírus, mas determina anticorpos contra a vacina, principalmente vacinas de mRNA e vetor viral. Com isso, após o início da vacinação, o estudo soroepidemiológico pode ser utilizado para avaliar a eficácia da vacina em produzir anticorpos nas pessoas (ROUTLEDGE *et al.*, 2022).

Entretanto, a coleta das amostras desta pesquisa aconteceu em período pré-vacinal (agosto a dezembro de 2020), por isso, o estudo soroepidemiológico foi uma estratégia pertinente para a compreensão do cenário epidemiológico naquele período.

Assim, os próximos parágrafos apresentam os pontos importantes do estudo sorológico para aquele período.

Em período pré-vacinal, o estudo soroepidemiológico por meio da realização testagem para a Covid-19 é fundamental para que se avalie e compreenda diversos fatores relacionados à doença, dentre elas: a resposta imune, o período da doença, a reatividade cruzada entre outros coronavírus, o período de imunidade pós-infecção, se a infecção protege contra infecções futuras e triagem para o atendimento clínico (CHENG *et al.*, 2020; LA MARCA *et al.*, 2020). Além de servir como norteador para tomadas de decisões em relação à saúde pública, porque com os estudos soroepidemiológicos pode-se compreender a disseminação do vírus, a evolução da pandemia e propor estratégias de prevenção (CAVATAIO; SCHNELL, 2021; LA MARCA *et al.*, 2020; PRAKASH *et al.*, 2021; TOZETTO-MENDOZA *et al.*, 2021).

Para que o estudo soroepidemiológico aconteça há necessidade de que os testes sejam eficientes e seguros. Existem dois tipos básicos de testes para a Covid-19, os testes virais que detectam o vírus e testes de anticorpos que são indiretos, porque determinam se o indivíduo foi anteriormente infectado (LA MARCA *et al.*, 2020). Em relação aos testes virais, são àqueles realizados através ensaios de reação em cadeia da polimerase de transcrição reversa (RT-PCR) usando amostras respiratórias, são considerados padrão ouro para o diagnóstico da doença (COTA *et al.*, 2020). Da mesma forma que testes de ensaios de imunoabsorção enzimática (ELISA) são considerados padrão ouro para testes imunológicos, além de apresentarem baixo custo e resultado relativamente rápido (HUERGO *et al.*, 2021).

Em relação as testes imunológicos, são mais frequentemente usados os testes de antígenos e de anticorpos. Para a detecção de anticorpos, utiliza-se os testes baseados em imunoensaios de fluxo lateral (LFIA) e ELISA, ou ainda imunoensaios quimioluminescentes (CLIA), todos estes tem a vantagem de poder apresentar resultado após a infecção por um período maior e, assim, realizar o estudo soroepidemiológico (COTA *et al.*, 2020; HUERGO *et al.*, 2021). Atualmente, no Brasil, os testes utilizando ELISA são seis: três detectando IgG, um IgM, um IgA e um IgA e IgM, o teste tendo como base IgG apresenta cerca de 95,8% de especificidade (COTA *et al.*, 2020). Em outro estudo, aponta a sensibilidade de IgG como 94,9%, assim, os testes IgG apresentam melhor desempenho quando comparados aos de IgM e IgA (TRÉ-HARDY *et al.*, 2021).

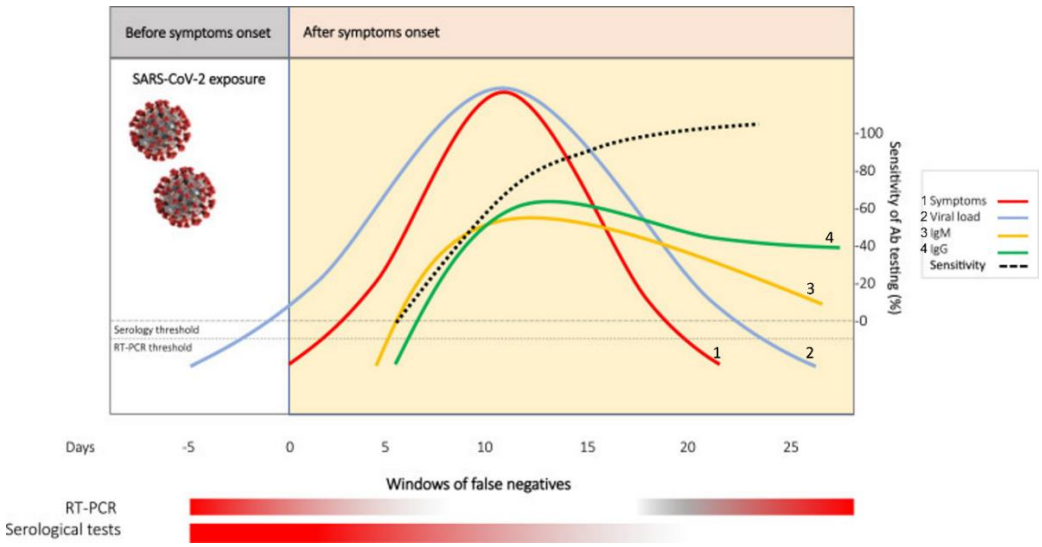
O teste ELISA indireto não apresenta reatividade cruzada com outros

coronavírus, em estudo realizando usando *western blot* em relação a proteína N, no entanto, apresentou reatividade cruzada entre anticorpos anti-N de *SARS-CoV* e *SARS-CoV-2* (PAN *et al.*, 2020; SHARMA *et al.*, 2021).

Para o uso de ELISA utiliza-se anticorpos da proteína N, porque ela induz a produção de anticorpos mais cedo do que a proteína S, a proteína N está relacionada com a replicação e montagem do vírion, por isso, permanece mais tempo ativa na célula (CHENG *et al.*, 2020; GUO *et al.*, 2020). Esse tipo de teste foi recomendado também porque possibilita realizar a triagem dos pacientes já recuperados para a doação do plasma convalescente e o monitoramento da resposta imune de vacinas candidatas ao combate da doença (THEEL *et al.*, 2020; TOZETTO-MENDOZA *et al.*, 2021).

Porém, para que o teste de ELISA seja utilizado, a amostra deve ser colhida 14 dias após a infecção, antes desse período a taxa de positividade apresenta-se baixa, as proteínas S1 e N nesse período foram as que apresentaram melhor desempenho para a detecção de antígenos (GILLOT *et al.*, 2020). Dessa maneira, a soroconversão é observada em maior parte entre os dias 5-7 e 14 dias após o início dos sintomas, assim, testes imunológicos realizados no período inicial da doença podem apresentar-se como falso-negativo, conforme apresenta-se na Figura 10, em que 0 marca o início dos sintomas por volta de 5 dias após a infecção (LA MARCA *et al.*, 2020).

Figura 10 - A relação temporal entre carga viral, sintomas e positividade em testes diagnósticos



Fonte: (LA MARCA *et al.*, 2020)

Legenda: A linha preta pontilhada no gráfico ilustra a sensibilidade do ensaio quimioluminescente como derivado da folha de dados de um teste comercial (Abbott Diagnostics, EUA). Os números apresentados na legenda e linhas não aparecem na imagem original, foram acrescentados para auxiliar a visualização.

Assim como mencionado no tópico anterior sobre os efeitos negativos na transmissibilidade e efetividade das vacinas com a variante Ômicron, há relatos de que ela pode também causar falhas nos testes que tem como alvo a proteína S, como os testes RT-PCR (KARIM; KARIM, 2021). Isso pode acontecer graças as diversas mutações que aconteceram na expressão dessa proteína (CALLAWAY, 2021).

2.7 SEROPREVALENCE OF SARS-COV-2 IN HOSPITALS WORKERS IN THE SOUTH REGION OF MINAS GERAIS STATE, BRAZIL: AN ANALYSIS IN THE PRE-VACCINE PERIOD

Seroprevalence of *SARS-CoV-2* in hospitals workers in the south region of Minas Gerais state, Brazil: An analysis in the pre-vaccine period

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Abstract

Determining the seroprevalence of SARS-CoV-2 infection can be performed by detecting anti-SARS-CoV-2 antibodies using immunoassays. The prevalence survey is essential to understand, through estimation, the development and transmission of the disease in the studied population. This study aimed to carry out a seroepidemiological survey of SARS-CoV-2 in hospital workers from three cities located in the south of the State of Minas Gerais, Brazil, in 2020. Enzyme-linked immunosorbent assays (ELISA) were performed on sera from 859 participants. The average age was 38 years. Women represented 71.4% and men 28.6%; health professionals were 74.5% and other workers in the administrative sector were 11.6%. The main symptoms reported by the participants were: fever 6.4%, difficulty breathing 5.8%, loss of smell and taste 7.0% and diarrhea 15.8%. 63.35% of the participants also reported contact with infected patients. 21.7% of the individuals presented positive ELISA tests, 62.7% were negative and 15.6% were undetermined. Hospital 3 had the highest positivity rate (22.9%), followed by hospital 2 (21.6%) and hospital 1 (20.3%) (p=0.079). Women had a higher prevalence positivity than men (22.8% and 18.7% respectively). The highest positivity in the test (22.0%) was observed among administrative professionals, followed by health professionals (20.9%). However, professionals who performed laboratory and imaging tests were more positive tests (30.3%), followed by administrative (22.6%), Covid hospital area (22.0%) and non-Covid hospital area (21.5%). A significant correlation was detected between positive ELISA tests and the following variables: previous serological tests, previous contact with infected patients, presence of fever, loss of smell and taste. It is concluded that the ELISA tests were, in the pre-vaccination period, important tools for the detection of antibodies against the virus among hospital professionals. the ELISA tests results showed no significant difference between hospital workers in different areas, nonetheless it demonstrated that hospital professionals in general are exposed to infection. Nevertheless, when analyzing the positive ELISA tests, there was a significant difference between reported symptoms and contact with patients with Covid-19.

Keywords: Covid-19; *SARS-CoV-2*; Seroepidemiology; Hospitals; ELISA; Pandemic.

1. Introduction

Coronavirus disease 2019 (Covid-19) is caused by *SARS-CoV-2* (Severe Acute Respiratory Syndrome-

Related Coronavirus 2) [1]. In December 2019, the virus was first identified in the city of Wuhan, China. In February 2020, the disease had its recognition by the World Health Organization (WHO) and in March 2020, the new coronavirus pandemic was declared [2]. After more than three years of transmission, on 07 December 2022, 641,487,094 cases had been recorded, with 6,621,419 global deaths [3]. In Brazil, on 08 December of the same year, 35,531,716 cases were confirmed with 690,677 deaths [4] and in Minas Gerais, on 07 December of the same year, 3,952,942 cases were confirmed with 64,038 deaths [5].

The *SARS-CoV-2* genome is made of single-stranded RNA [6]. The virus has four structural proteins: Spike (S), Envelope (E), Membrane (M) and Nucleocapsid (N). The virus' attachment and entry into the host cell occurs through binding of the viral S protein and the cellular receptor for conservative enzyme angiotensin 2 (ACE-2) [7]. *SARS-CoV-2* is transmitted and spread through airways via aerosols and also by droplets of saliva released when talking, coughing or sneezing. The virus can infect and early replicate in the throat during infection. Therefore, the use of protective measures such as masks, contact tracing and social distancing is necessary to prevent the infection's spread [8]. When infected, an individual can be asymptomatic or develop a symptomatic disease.

It is important to notice that asymptomatic cases represent great epidemiological relevance, because asymptomatic patients are potential transmitters of the virus. In turn, symptomatic people are considered more contagious [9,10]. Symptoms begin 2 to 14 days after exposure to the virus [11]. Symptoms such as fever are common, affecting more than 80% of Covid patients, coughing affects between 50-80% and dyspnea affects about 30% of patients. Mild symptoms may cease after the first week of infection, but it may be persistent or progress to severe stages of the disease [12–14]. Individuals who develop mild symptoms usually have no changes in chest images. Patients with moderate symptoms present more intense clinical manifestations and alterations in chest images similar to mild pneumonia [15]. Patients who develop severe symptoms may experience rapid disease progression with respiratory failure that may need mechanical ventilation, organ failure, and intense care unit treatment. Severe lung damage is caused by immune response against *SARS-CoV-2*. Due to excess of secreted inflammatory cytokines, it can progress to systemic inflammation. Accordingly, to the disease's severity, other systems can be affected [12]. The disease can be aggravated by comorbidities, such as renal, cerebrovascular, respiratory, and cardiovascular diseases, hypertension, chronic obstructive pulmonary disease, obesity and diabetes. These conditions were associated with fatal outcomes, and they were recognized as risk factor for severe Covid-19 [10].

To diagnose the disease, laboratory imaging, molecular and serological tests can be carried out, in addition to clinical examination in situations of lack of tests. RT-PCR assays performed with respiratory samples is considered the gold standard for the diagnosis at early infection [16]. Regarding serological tests, antigen and antibody tests are more frequently used. For detection of antibodies, tests based on lateral flow immunoassays, enzyme-linked immunosorbent assays (ELISA) and chemiluminescent immunoassays are used. All of these are suitable for seroepidemiological studies and they have great value to understand disease transmission in a specific region or community [17]. These tests make it also possible to screen patients for donation of convalescent plasma and to monitor the immune response induced by infection or vaccination [18].

ELISA tests are used to detect *SARS-CoV-2* IgG, IgM and IgA antibodies, and the detection of IgG and IgA antibodies is performed better when compared to IgM [19]. The specificity of these tests is about 95.8% [17]. Seroconversion is observed in most patients between the 5th and 14th day after the onset of symptoms. Generally, ELISA tests use the virus' N protein as the antigen, since it induces production of antibodies earlier than the S protein [20].

Testing for Covid-19 is fundamental to assess and understand several factors related to the disease, such as the immune response, the disease's stage, cross-reactivity among other coronaviruses, the post-infection period immunity and the disease screening. Besides serving as a guide for decision-making in relation to public health, contacts can be traced and thus, with the results of seroepidemiological studies, it's possible to understand not just how the virus spreads, the pandemic evolution and with that data, control measures can be planned and applied. Moreover, seroepidemiological studies demonstrate the number of infected people in a sample space, which can reveal the disease's evolution in an specific epidemiological scenario [21].

With these considerations, the objective of this work is to carry out a seroepidemiological survey of *SARS-CoV-2*, in hospitals in the south of Minas Gerais state, Brazil. ELISA was performed through the quantification of IgG antibodies against nucleocapsid protein of *SARS-CoV-2* in a period before the beginning of vaccination, that was a critical period for the virus' transmission and with the state and the municipal decrees restricting the movement of people.

2 Material and Methods

2.1 Study and sampling site

This study was conducted in three hospitals located in the south of Minas Gerais, Brazil. This region concentrates a significant people's movement between cities for professional reasons, as well as for health treatment, whereas there are regional health services at these surveyed places that cover a lot of cities there. Three selected hospitals were chosen based on the Covid-19 healthcare: Santa Casa de Misericordia de Alfenas (hereinafter referred to as hospital 1), UNIMED in Poços de Caldas (hereinafter referred to as hospital 2), and Field hospital in Varginha (hereinafter referred to as hospital 3).

Professionals who were directly and indirectly involved with Covid-19 combat strategies were invited to participate. Sampling was determined by a statistical test according to Medronho [22] for each institution-based professionals' number working in the hospital. The measurement of the sample size was performed for each institution based on the number of professionals working in the hospital in a simple random sampling study. The following inclusion criteria were used for sample collection: a) all health professionals directly involved with suspected or confirmed individuals with Covid-19. b) professionals who worked in other sectors.

Individuals were invited to participate in a voluntary way, through a previous explanation of the objectives, benefits, and the confidentiality of their identities. Each participant was asked to sign an informed consent formulary. After this consent, a questionnaire was applied to collect data on age, gender, previous tests to detect *SARS-CoV-2* (date and kind of test performed, result), symptoms (if tested positive, what symptoms were detected), previous contact with people who had a confirmed diagnosis of Covid-19, presence of comorbidities (diabetes, high blood pressure, bronchitis, asthma, etc) and a regular medication intake. 3.5 mL of venous blood was collected from each participant to obtain serum. The collect period took place between August and December 2020. Variants of SARS-CoV-2 that were registered in south of Minas Gerais during the studied period were B.1.1.28, B.1.1.33, B.1.1, P.2 (FIOCRUZ, 2022).

This work was carried out in a previous period of the vaccination for *SARS-CoV-2*, within the quarantine, when people were isolated and followed strict state transitory rules, as the obligatory use of masks and hygiene criteria.

2.2 Ethical aspects

This work follows all norms and laws that regulate the human material use, according to the criteria of Resolution 466 of the National Health Council and its complementary norms and resolutions that was approved by the Research Ethics Committee of the Federal University of Alfenas under the protocol 33623320.2.0000.5142.

2.3 Enzyme immunoassay (ELISA)

Serum samples were initially tested for the detection of IgG anti-*SARS-CoV-2* antibodies using an enzyme-linked immunosorbent assay (ELISA) developed by the Vaccine Technology Center of the Federal University of Minas Gerais [24]. The test detected antibodies against the Nucleocapsid (N) protein of *SARS-CoV-2*. Nunc MaxiSorp® 96-well microtiter plates (eBIOSCIENCE, USA) were used to perform the ELISA. The wells were coated with 100 µL of N protein solution (0.4 mg/mL) diluted in carbonate-bicarbonate buffer (0.1 M pH 9.6) and the plate incubated for 16 hours at 4 °C. Then, the plate was washed for three times with buffered saline solution (PBS) containing 0.05% Tween 20 (PBS-T) and then blocked with a 5% solution of skimmed milk powder (Molico® Nestlé, Brazil) in PBS-T for 2 hours at 37°C. Then, the plate was washed for six times with PBS-T, 100 µL of the test sera were added at a dilution of 1:100. The plate was incubated for 1 hour at 37°C. After this period, the plate was washed again for four times with PBS-T, 100 µL of anti-human IgG peroxidase conjugate (Sigma-Aldrich, USA) diluted 1:5000 was added. The plate was incubated for 1 hour at 37°C. Then, the plate was washed for four times with PBS-T and then an addition of 100 µL per well of a solution of O-Phenylenediamine Dihydrochloride (OPD) (Sigma-Aldrich, USA) in citrate-phosphate buffer pH 4.5 with 0.08% H2O2 was done. After the addition of the solution, the plate was incubated for 15 minutes at room temperature, protected from light, after that the optical densities were read at 450 nm on an Anthos Zenyth 200rt microplate reader (Biochrom, UK). The cut-off value was defined as the mean of the absorbance readings of the negative control (sera collected from *SARS-CoV-2* negative individuals by serological and molecular tests) plus three times the standard deviation. As positive controls for the ELISA tests, sera with a positive diagnosis for *SARS-CoV-2* through molecular and serological assays were used. To determine the diagnostic value, an index was established. The index value was obtained by dividing the average optical absorption value by the cutoff value. Index values lower than 0.80 were

considered negative. Index values between 0.81 and 1.09 were considered indeterminate. Values equal to or higher than 1.10 were considered positive. In case of an indeterminate result occurrence, the sample was reanalyzed.

2.4 Statistical analysis

Statistical analyzis were performed using SPSS v.20 software, considering the percentages and valid values of each hospital, as well as the relationship between the studied variables, using chi-square or Fisher's exact tests, all considered at a level of 5% significance.

For the analysis of age, the participants were divided in groups considering a 10 years interval between ages (for example, 18-27 years old), according to a study carried out in Ireland that evaluated hospital professionals [25]. Also, participants were divided in groups considering the hospital they worked for (supplementary table 1A) and ELISA test result (supplementary table 1B).

3 Results

In this work, 859 individuals were selected to survey the prevalence of Covid-19 through the detection of IgG antibodies against the Nucleocapsid protein of SARS-CoV-2 in hospitals located in the cities of Alfenas, Poços de Caldas and Varginha, located in south of Minas Gerais, Brazil. The mean age of the participants was 38.43 years +/- 10.025 standard deviation (SD) (standard deviation index 1.25), being 18 years the lowest and 72 years the highest. The mean age at hospital 1 (Alfenas) was 38.33 +/- 10.00 SD, at hospital 2 (Poços de Caldas) it was 37.68 +/- 10.278 SD and at hospital 3 (Varginha) it was 39.00 +/- 9.876 SD.

The largest number of participants at the 28 to 37 years old group (290 participants, 33.8%), followed by the age group of 38 to 47 years old (259 participants, 30.2%), the group of 48 to 59 years old (131 participants, 15.3%), the group of 18 to 27 years old (119 participants, 13.9%), the ones that are 58 to 67 years old (26 participants, 3.0%) and 31 participants (3.6%) did not inform their age ($p < 0.001$) (Supplementary Table 1A).

Table 1 shows the distribution by gender, area of profession, sector of work, previously tests performed, type of test performed, test result, symptoms, previous contact with the patient, prior morbidity to Covid-19 (presence of comorbidities), medication schedule and ELISA test results at each participating hospital unit. Data are presented by analysis of the total value and the percentage of the sample and by participating hospital units.

Regarding gender, 28.6% (246) of the participants were men and 71.4% (613) were women, considering the total number of participants ($p<0.001$). When analysed by hospital, the highest percentage of women was in hospital 1 (74.0%, 222), followed by hospital 2 (73.7%, 171) and hospital 3 (67.3%, 220) ($p=0.017$); and for men it was at hospital 3 (32.7%, 107), followed by hospital 2 (26.3%, 61) and hospital 1 (26.0%, 78) ($p=0.001$).

About profession, 74.5% (640) of the participants were included as health professionals (doctors, nurses, physiotherapists, etc.) who provided direct or indirect care to patients with Covid-19. Considering the distribution of participants by hospital unit, the highest percentage was obtained in hospital 1 with 78.0% (234), followed by hospital 2 with 77.2% (179) and hospital 3 with 69.4% (227) ($p=0.015$); considering the other sectors, 11.6% (100) were professionals in the administrative area (secretaries, attendants, drivers, cleaning assistants, etc.). When the distribution of hospital unit of this group was analysed, it was observed that the highest percentage of these professionals was in hospital 3 with 16.5% (54), followed by hospital 1 with 9.3% (28) and hospital 2 with (7.8 % (18) ($p<0.001$); In addition to the health and the administrative area, 4.2% (36) of the participants reported having other professions and 9.7% (83) of the participants did not inform the profession. When they were evaluated by sector of work, 17.5% (150) of the participants were classified as a Covid-19 hospital, with the highest percentage of professionals in hospital 1 with 25.7% (77), followed by hospital 3 with 14.7% (48) and hospital 2 with 10.8% (25) ($p<0.001$); In turn, 58.0% (498) of the participants were in the non-Covid-19 hospital sector, with the highest percentage in hospital 2 with 72.4% (168), followed by hospital 1 with 58.7% (176) and hospital 3 with 47.1% (154) ($p=0.474$); 7.2% (62) of the participants were in the administration sector, with the highest percentage in hospital 2 with 13, 4% (31), followed by hospital 3 with 5.5% (18) and hospital 1 with 4.3% (13) ($p=0.015$); 3.8% (33) of the participants worked in the sector of imaging tests and laboratories, with the highest percentage in hospital 3 with 8.0% (26), followed by hospital 2 with 1.7% (4) and hospital 1 with 1.0% (3) ($p=0.001$); 2.7% (23) of the participants were classified in other sectors and 10.8% (93) did not inform which sector they worked.

The participants were asked if they had performed a Covid-19 test prior to the blood collection, which test was performed and the test result, 66.5% (571) reported having performed tests prior to the blood collection and 32.5% (279) did not have performed, while 1% (9) of the participants were unable to say or did not respond

(p<0.001). At about the participants that submit themselves to previous tests, the highest percentage was from hospital 2 (90.1%, 209), followed by hospital 1 (89.3%) and hospital 3 (28.7%) (p<0.001).

Considering type of test, 9.9% (85) of the participants reported having performed molecular tests for detection of the virus (RT-PCR), 47.1% (405) performed serological tests, 4.7% (40) both molecular and serological tests and 38.3% (329) did not respond (p<0.001). Analysing the percentage of RT-PCR tests performed per hospital, hospital 2 had the highest percentage (19.4%, 45), followed by hospital 3 (8.9%, 29) and hospital 1 (3.7%, 11) (p=0.001); regarding serological tests, the highest percentage was from hospital 1 (73.3%, 220), followed by hospital 2 (59.1%, 137) and hospital 3 (14.7%, 48) (p<0.001) ; and both tests the highest percentage was from hospital 2 (7.8%, 18), followed by hospital 1 (3.7%, 11) and hospital 3 (3.4%, 11) (p=0.294).

In relation to results of the tests performed previously for the detection of Covid-19, 5.4% (46) of the participants reported a positive result, 59.5% (511) reported a negative result, 0.2% (2) reported an undetermined result and 34.9% (329) did not know or did not answer (p<0.001). Analysing the percentage of test results by hospital, the highest percentage of positive results was in hospital 1 (6.3%, 19), followed by hospital 3 (6.1%, 20) and hospital 2 (3.0%, 7) (p=0.033).

When the presence of typical signs and symptoms of Covid-19 was investigates, it was observed that 6.4% (55) of the participants reported fever. Analysing this information by hospital, hospital 1 had the highest percentage (10.0%, 30), followed by hospital 3 (5.5%, 18) and hospital 2 (3.0%, 7) (p<0.001); 5.8% (50) of the participants reported breathing difficulty, hospital 1 demonstrated the highest percentage (8.0%, 24), followed by hospital 3 (5.2%, 17) and hospital 2 (3.9% , 9) (p=0.034); loss of smell and taste was reported by 7% (60) of the participants, with the highest percentage in hospital 1 (8.3%, 25), followed by hospital 3 (7.3%, 24) and hospital 2 (4 .7%, 11) (p=0.057); episodes of diarrhea was reported by 15.8% (136) of the participants, with the highest percentage found in hospital 1 (17.0%, 51), followed by hospital 2 (15.9%, 37) and hospital 3 (14.7%, 48) (p=0.302).

Previous contact with symptomatic people for Covid-19 was evaluated, which may have occurred inside and outside of the work units, 63.3% (544) reported contact with positive people, 30.3% (260) said that they did not have contact with sick people and 6.4% (55) did not answer or did not know if this contact happened (p<0.001). The highest percentage of people in contact with patients with the disease was from hospital 1 (74.0%, 222), followed by hospital 2 (65.5%, 152) and hospital 3 (52.0%, 170) (p <0.001).

Another important aspect is to understand whether the participants had morbidity prior to Covid-19. These factors are associated with greater severity of the disease. 9.3% (80) of participants reported having hypertension, 4.0% (34) bronchitis, 2.2% (19) asthma, 1.7% (15) diabetes, 1.0% (9) having bronchitis and high blood pressure and 0.9% (8) diabetes and high blood pressure and 80.7% (693) of the participants did not answer or did not know if they had any health problems (p<0.001).

Hypertension morbidity had a higher percentage in hospital 1 (11.7%, 35), followed by hospital 3 (10.4%, 34) and hospital 2 (4.7%, 11) (p<0.001). Regarding bronchitis, the highest percentage was in hospital 1 (6.0%, 18), followed by hospital 2 (3.4%, 8) and hospital 3 (2.4%, 8) (p=0.053). Regarding asthma, hospital 3 had the highest percentage (3.1%, 10), followed by hospital 2 (2.2%, 5) and hospital 1 (1.3%, 4) (p=0.196). Diabetes morbidity had a higher percentage in hospital 1 (2.3%, 7), followed by hospital 3 (1.5%, 5) and hospital 2 (1.3%, 3) (p=0.449). The highest percentage of the prevalence of bronchitis and high blood pressure was in hospital 3 (2.4%, 8), followed by hospital 1 (0.3%, 1) and none in hospital 2 (0.0%, 0) (p=0.020). And the highest percentage regarding the prevalence of diabetes and high blood pressure was in hospital 2 (1.3%, 3), followed by hospital 3 (0.9%, 3) and hospital 1 (0.7%, 2) (p=0.882).

When asked about the regular use of medication, 62.9% (540) participants reported they did not use medication. 9.5% (82) reported using drugs for hypertension, 2.3% (20) reported they use medication to treat diabetes, 0.8% (7) use medication to treat the heart and 24.4% (210) reported using other types of medication (p<0.001). The highest percentage of hypertensive individuals was registered in hospital 3 (11.3%, 37), followed by hospital 1 (11.0%, 33) and hospital 2 (5.2%, 12) (p=0.001). In relation to the use of medication to control morbidities, it was noticed that the medication to control diabetes was a higher percentage in hospital 1 (3.3%, 10), followed by hospital 3 (2.1%, 7) and hospital 2 (1.3%, 3) (p=0.157). The use of medication for treating the heart had a higher percentage in hospital 2 (1.3%, 3), followed by hospital 1 (0.7%, 2) and hospital 3 (0.6%, 2) (p=0.867). And the other types of medication had a higher percentage in hospital 1 (25.0%, 75), followed by hospital 2 (24.6%, 57) and hospital 3 (23.9%, 78) (p=0.158).

Regarding the result of the ELISA test, 21.6% (186) of the individuals had a positive result, 62.7% (539)

had a negative result and 15.6% (134) had an indeterminate result (p<0.001). Hospital 3 (Hospital de Campanha de Varginha) had the highest percentage of positivity (22.9%, 75), followed by Hospital 2 (Unimed Hospital in Poços de Caldas) (21.6%, 50) and Hospital 1 (Santa Casa de Alfenas) (20.3%, 61) (p=0.079).

At about the ELISA result analysed by age, the highest positivity was in the age group of 38-47 years old with 60 participants (32.3%), followed by the age group between 28-37 years old with 55 participants (29.6%), 48-57 years with 27 participants (14.5%), 18-27 years with 25 participants (13.4%) and 58-67 years with 10 participants (5.4%), in relation to a group of 68-77 years there were no positive cases and 9 positive cases from participants who did not inform their age (p<0.001) (Supplementary Table 1B).

Once the sample was characterized, the variables studied were associated with positivity in the ELISA test, in order to assess which public was more vulnerable to the virus infection, as well as which reported symptoms were related to the infection. Table 2 shows the distribution of positivity in the ELISA test regarding the parameters previously analysed in Table 1, analysing the value and percentage of the result and individually by variable group.

Regarding the distribution by gender in relation to the ELISA test, a higher prevalence was observed in women (75.3%, 140) with a positive test compared to men (24.7%, 46) (p<0.001). And when evaluating positivity within the group of variables, the percentage of women continued to be higher (22.8%) compared to men (18.7%) (p=0.537).

Referring to the distribution by profession, 82.2% (134) of the participants from the health area were positive in the test, 13.5% (22) of the participants were from the administrative area and 4.3% (7) of the participants were from other professions (p<0.001). When evaluating the percentage of the variable groups, the highest percentage was among professionals in the administrative area (22.9%), followed by the health area (20.0%) and other areas (19.4%) (p=0.893).

Concerning the distribution by sector of work, the participants were positive in the test: 63.7% (107) of the participants did not work directly with Covid-19 (non-Covid hospital) reported more positivity in the test, while 19.6% (33) of participants working in areas directly related to treating patients with Covid-19 had a positive ELISA test. 8.3% (14) of the participants reported working in administrative areas, as well as 6.0% (10) were professionals in the sector of diagnosis by imaging or laboratory tests and 2.4% (4) professionals who worked in other areas (p<0.001).

However, when evaluating the work sector as a variable, the highest percentage was of professionals who performed imaging and laboratory tests (30.3%), followed by the administrative sector (22.6%), workers with direct contact with patients with Covid -19 (22.0%), workers who did not work directly with patients with Covid-19 (21.5%) and other sectors (17.4%) (p=0.433).

The comparison between the positive result of the ELISA tests with the diagnostic tests previously performed by the participants was 75.4% (89) of the participants regarding the serological tests, 17.8% (21) regarding the RT-PCR and 6.8% (8) for both tests (p<0.001). Analysing the test performed by variable, the highest percentage was in relation to RT-PCR (24.7%), followed by serological tests (22.0%) and both tests (20.0%) (p=0.753).

When the data from positive ELISA tests and the presence of signs and symptoms for Covid-19 were crossed, 10.3% (19) had fever, 9.2% (17) respiratory difficulty, 18.4% (34) loss of smell and taste and 21.1% (39) diarrhea (p=0.004). The last one was the most common symptom among the positive participants.

However, when evaluating the crossing between positive ELISA test and the presence of signs and symptoms for Covid-19 as a variable, the highest percentage was in the loss of smell and taste (56.7%), followed by fever (34. 5%), respiratory distress (34.0%) and diarrhea (28.7%) (p=0.007).

When comparing positivity in the ELISA test and previous contact with symptomatic people, 67.1% (116) reported contact and had a positive test, 32.9% participants (57) did not know or did not have contact with infected people (p<0.001), but these participants had a positive result in the ELISA tests, which shows that they may have been contaminated by asymptomatic people. However, when evaluating the percentage as a variable, the highest percentage of positive cases was from participants who did not know or had no contact with positive people (21.9%) followed by people who had contact with positive people (21.3%) (p<0.001).

When comparing positivity in the ELISA test and morbidity prior to Covid-19, 51.3% (20) reported having hypertension, 23.1% (9) bronchitis, 10.3% (4) asthma, 7.7% (3) diabetes and the association between bronchitis and high blood pressure, and the association between diabetes and high blood pressure and other types of diseases, no positive cases were recorded (p<0.001). However, when analysing this association as a variable, the highest percentage was in relation to the association between bronchitis and hypertension (33.3%), bronchitis

(26.5%), hypertension (25.0%), asthma (21.1%) and diabetes (20.0%), and the association between diabetes and high blood pressure and other types of diseases, no positive cases were recorded (p=0.365).

When comparing positivity in the ELISA test and medication intake, 29.4% (20) took medication for hypertension, 5.9% (4) took medication to control diabetes, 1.5% (1) took medication for the heart and 63.2% (43) of other types of medication unrelated to comorbidities that increase the risk of severe disease (p<0.001). In the same way, when evaluating this association by variables, the highest percentage was related to the use of drugs for hypertension (29.4%), other drugs (20.5%), drugs to control diabetes (20.0%) and heart medications (14.3%) (p=0.445).

The association between the positivity of the ELISA tests and the studied variables showed a statistically significant value for the positive result in the tests previously performed in the three hospitals (p=0.011; 0.016 and <0.001), for the loss of smell and taste in two hospitals (p<0.001 and <0.001) and for the presence of fever reported in only one hospital (p=0.03) (Table 3).

Table 1: Distribution of the number and percentage of participants in each hospital unit in relation to gender, area of profession, sector of work, tests previously performed, type of test, test result, symptoms common to Covid-19, patient contact, disease prevalence, regular medication use, and ELISA test result.

	Hospital unit								
	Hospital 1		Hospital 2		Hospital 3		Total		
Gender	Number (%)	p-value	Number (%)	p-value	Number (%)	p-value	Number (%)	p-value	p-value of the lines
Male	78 (26,0%)	<0,001	61 (26,3%)	<0,001	107 (32,7%)	<0,001	246 (28,6%)	<0,001	0,001
Female	222 (74,0%)		171 (73,7%)		220 (67,3%)		613 (71,4%)		0,017
Total	300 (100,0%)		232 (100,0%)		327 (100,0%)		859 (100,0%)		<0,001
Profession area									
Health	234 (78,0%)	<0,001	179 (77,2%)	<0,001	227 (69,4%)	<0,001	640 (74,5%)	0,001	0,015
Administrative	28 (9,3%)		18 (7,8%)		54 (16,5%)		100 (11,6%)		<0,001
Others	1 (0,3%)		7 (3,0%)		28 (8,6%)		36 (4,2%)		<0,001
Uninformed	37 (12,3%)		28 (12,1%)		18 (5,5%)		83 (9,7%)		0,038
Total	300 (100,0%)		232 (100,0%)		327 (100,0%)		859 (100,0%)		<0,001
Work sector									
Covid Hospital	77 (25,7%)	<0,001	25 (10,8%)	<0,001	48 (14,7%)	<0,001	150 (17,5%)	<0,001	<0,001
Non-Covid Hospital	176 (58,7%)		168 (72,4%)		154 (47,1%)		498 (58,0%)		0,474
Administration	13 (4,3%)		31 (13,4%)		18 (5,5%)		62 (7,2%)		0,015
Image/Lab exam	3 (1,0%)		4 (1,7%)		26 (8,0%)		33 (3,8%)		<0,001
Others	0 (0,0%)		0 (0,0%)		23 (7,0%)		23 (2,7%)		no result
Uninformed	31 (10,3%)		4 (1,7%)		58 (17,7%)		93 (10,8%)		<0,001
Total	300 (100,0%)		232 (100,0%)		327 (100,0%)		859 (100,0%)		<0,001
If tests were performed previously									
yes	268 (89,3%)	<0,001	209 (90,1%)	<0,001	94 (28,7%)	<0,001	571 (66,5%)	<0,001	<0,001
No	26 (8,7%)		22 (9,5%)		231 (70,6%)		279 (32,5%)		<0,001
Uninformed	6 (2,0%)		1 (0,4%)		2 (0,6%)		9 (1,0%)		0,097
Total	300 (100,0%)		232 (100,0%)		327 (100,0%)		859 (100,0%)		<0,001
Type of test performed previously									
RT-PCR	11 (3,7%)	<0,001	45 (19,4%)	<0,001	29 (8,9%)	<0,001	85 (9,9%)	<0,001	<0,001
Serological	220 (73,3%)		137 (59,1%)		48 (14,7%)		405 (47,1%)		<0,001
Both	11 (3,7%)		18 (7,8%)		11 (3,4%)		40 (4,7%)		0,294

Uninformed		58 (19,3%)		32 (13,8%)		239 (73,1%)		329 (38,3%)		<0,001
Total		300 (100,0%)		232 (100,0%)		327 (100,0%)		859 (100,0%)		<0,001
Test result performed										
Positive		19 (6,3%)		7 (3,0%)		20 (6,1%)		46 (5,4%)		0,033
Negative		240 (80,0%)	<0,001	199 (85,8%)	<0,001	72 (22,0%)	<0,001	511 (59,5%)	<0,001	<0,001
Undetermined		1 (0,3%)		0 (0,0%)		1 (0,3%)		2 (0,2%)		1
Uninformed		40 (13,3%)		26 (11,2%)		234 (71,6%)		300 (34,9%)		<0,001
Total		300 (100,0%)		232 (100,0%)		327 (100,0%)		859 (100,0%)		<0,001
Symptoms										
Fever	Yes	30 (10,0%)		7 (3,0%)		18 (5,5%)		55 (6,4%)		<0,001
	no	269 (89,7%)	<0,001	225 (97,0%)	<0,001	309 (94,5%)	<0,001	803 (93,5%)	<0,001	0,001
	Uninformed	1 (0,3%)		0 (0,0%)		0 (0,0%)		1 (0,1%)		no result
	Total	300 (100,0%)		232 (100,0%)		327 (100,0%)		859 (100,0%)		<0,001
Breathing difficulty	Yes	24 (8,0%)		9 (3,9%)		17 (5,2%)		50 (5,8%)		0,034
	No	275 (91,7%)	<0,001	223 (96,1%)	<0,001	301 (92,0%)	<0,001	808 (94,1%)	<0,001	0,003
	Uninformed	1 (0,3%)		0 (0,0%)		0 (0,0%)		1 (0,1%)		no result
	Total	300 (100,0%)		232 (100,0%)		327 (97,2%)		859 (100,0%)		<0,001
Loss of smell and taste	Yes	25 (8,3%)		11 (4,7%)		24 (7,3%)		60 (7,0%)		0,057
	No	274 (91,3%)	<0,001	221 (95,3%)	<0,001	303 (92,7%)	<0,001	798 (92,9%)	<0,001	0,002
	Uninformed	1 (0,3%)		0 (0,0%)		0 (0,0%)		1 (0,1%)		no result
	Total	300 (100,0%)		232 (100,0%)		327 (100,0%)		859 (100,0%)		<0,001
Diarrhea	Yes	51 (17,0%)		37 (15,9%)		48 (14,7%)		136 (15,8%)		0,302
	No	248 (82,7%)	<0,001	195 (84,1%)	<0,001	279 (85,3%)	<0,001	722 (84,1%)	<0,001	<0,001
	Uninformed	1 (0,3%)		0 (0,0%)		0 (0,0%)		1 (0,1%)		no result
	Total	300 (100,0%)		232 (100,0%)		327 (100,0%)		859 (100,0%)		<0,001
Contact with Covid-19 patient										
Yes		222 (74,0%)		152 (65,5%)		170 (52,0%)		544 (63,3%)		<0,001
No		54 (18,0%)	<0,001	75 (32,3%)	<0,001	131 (40,1%)	<0,001	260 (30,3%)	<0,001	<0,001
Uninformed		24 (8,0%)		5 (2,2%)		26 (8,0%)		55 (6,4%)		<0,001
Total		300 (100,0%)		232 (100,0%)		327 (100,0%)		859 (100,0%)		<0,001
Disease prevalence										
Diabetes		7 (2,3%)	<0,001	3 (1,3%)	<0,001	5 (1,5%)	<0,001	15 (1,7%)	<0,001	0,449
Bronchitis		18 (6,0%)		8 (3,4%)		8 (2,4%)		34 (4,0%)		0,053

Hypertension	35 (11,7%)		11 (4,7%)		34 (10,4%)		80 (9,3%)	<0,001
Asthma	4 (1,3%)		5 (2,2%)		10 (3,1%)		19 (2,2%)	0,196
Diabetes and hypertension	2 (0,7%)		3 (1,3%)		3 (0,9%)		8 (0,9%)	0,882
Bronchitis and hypertension	1 (0,3%)		0 (0,0%)		8 (2,4%)		9 (1,0%)	0,020
Others	0 (0,0%)		0 (0,0%)		1 (0,3%)		1 (0,1%)	no result
Uninformed	233 (77,7%)		202 (87,1%)		258 (78,9%)		693 (80,7%)	0,033
Total	300 (100,0%)		232 (100,0%)		327 (100,0%)		859 (100,0%)	<0,001
Regular use of medication								
For Hypertension	33 (11,0%)		12 (5,2%)		37 (11,3%)		82 (9,5%)	0,001
For diabetes	10 (3,3%)		3 (1,3%)		7 (2,1%)		20 (2,3%)	0,157
For heart	2 (0,7%)	<0,001	3 (1,3%)	<0,001	2 (0,6%)	<0,001	7 (0,8%)	<0,001	0,867
Others	75 (25,0%)		57 (24,6%)		78 (23,9%)		210 (24,4%)	0,158
Uninformed	180 (60,0%)		157 (67,7%)		203 (62,1%)		540 (62,9%)	0,053
Total	300 (100,0%)		232 (100,0%)		327 (100,0%)		859 (100,0%)	<0,001
ELISA test result								
Positive	61 (20,3%)		50 (21,6%)		75 (22,9%)		186 (21,7%)	0,079
Negative	199 (66,3%)	<0,001	138 (59,5%)	<0,001	202 (61,8%)	<0,001	539 (62,7%)	<0,001	<0,001
Undetermined	40 (13,3%)		44 (19,0%)		50 (15,3%)		134 (15,6%)	0,567
Total	300 (100,0%)		232 (100,0%)		327 (100,0%)		859 (100,0%)	<0,001

Table 2: Distribution of the ELISA test result in relation to gender, area of profession, sector of work, tests previously performed, type of test, test result, symptoms common to Covid-19, patient contact, disease prevalence and regular use of medicines.

	ELISA test															
	Positive				Negative				Undetermined				Total			
	Number (%)	p-value	% group	p-value	Number (%)	p-value	% group	p-value	Number (%)	p-value	% group	p-value	Number (%)	p-value	p-value of the lines	% p-value of the lines
Gender																
Male	46 (24,7%)	<0,001	18,70%	<0,001	163 (30,2%)	<0,001	66,30%	0,657	37 (27,6%)	<0,001	15,00%	0,857	246 (100,0%)	<0,001	<0,001	<0,001
Female	140 (75,3%)		22,80%		376 (69,8%)		61,30%		97 (72,4%)		15,80%		613 (100,0%)		<0,001	<0,001
Total (absolute number and %)	186 (100,0%)				539 (100,0%)				134 (100,0%)				859 (100,0%)		<0,001	
Profession area																
Health	134 (82,2%)		20,90%		410 (82,7%)		64,10%		96 (82,1%)		15,00%		640 (100,0%)		<0,001	<0,001
Administrative	22 (13,5%)	<0,001	22,00%	0,893	60 (12,1%)	<0,001	60,00%	0,565	18 (15,4%)	<0,001	18,00%	0,146	100 (100,0%)	<0,001	<0,001	<0,001
Others	7 (4,3%)		19,40%		26 (5,2%)		72,20%		3 (2,6%)		8,30%		36 (100,0%)		<0,001	<0,001
Total (absolute number and %)	163 (100,0%)				496 (100,0%)				117 (100,0%)				776 (100,0%)		<0,001	
Work sector																
Covid Hospital	33 (19,6%)		22,00%		98 (20,4%)		65,30%		19 (16,2%)		12,70%		150 (100,0%)		<0,001	<0,001
Non-Covid Hospital	107 (63,7%)		21,50%		311 (64,7%)		62,40%		80 (68,4%)		16,10%		498 (100,0%)		<0,001	<0,001
Administration	14 (8,3%)	<0,001	22,60%	0,433	35 (7,3%)	<0,001	56,50%	0,635	13 (11,1%)	<0,001	21,00%	0,108	62 (100,0%)	<0,001	<0,001	<0,001
Image/Lab exam	10 (6,0%)		30,30%		20 (4,2%)		60,60%		3 (2,6%)		9,10%		33 (100,0%)		<0,001	<0,001
Others	4 (2,4%)		17,40%		17 (3,5%)		73,90%		2 (1,7%)		8,70%		23 (100,0%)		<0,001	<0,001
Total (absolute number and %)	168 (100,0%)				481 (100,0%)				117 (100,0%)				766 (100,0%)		<0,001	
Type of test performed previously																
RT-PCR	21 (17,8%)	<0,001	24,70%	0,753	49 (14,9%)	<0,001	57,60%	0,873	15 (17,9%)	<0,001	17,60%	0,838	85 (100,0%)	<0,001	<0,001	<0,001

Serologic		89 (75,4%)		22,00%		254 (77,4%)		62,70%		62 (73,8%)		15,30%		405 (100,0%)		<0,001	<0,001
Both		8 (6,8%)		20,00%		25 (7,6%)		62,50%		7 (8,3%)		17,50%		40 (100,0%)		<0,001	<0,001
Total (absolute number and %)		118 (100,0%)				328 (100,0%)				84 (100,0%)				530 (100,0%)		<0,001	
*** Symptoms**																	
Fever	Yes	19 (10,3%)	<0,001	34,50%	0,061	30 (5,6%)	<0,001	54,50%	0,461	6 (4,5%)	<0,001	10,90%	0,336	55 (100,0%)	<0,001	<0,001	<0,001
	No	166 (89,7%)		20,70%		509 (94,4%)		63,40%		128 (95,5%)		15,90%		803 (100,0%)		<0,001	<0,001
Total (absolute number and %)		185 (100,0%)				539 (100,0%)				134 (100,0%)				858 (100,0%)		<0,001	
Breathing difficulty	Yes	17 (9,2%)	<0,001	34,00%	0,080	27 (5,0%)	<0,001	54,00%	0,405	6 (4,5%)	<0,001	12,00%	0,450	50 (100,0%)	<0,001	0,001	<0,001
	No	168 (90,8%)		20,80%		512 (95,0%)		63,40%		128 (95,5%)		15,80%		808 (100,0%)		<0,001	<0,001
Total (absolute number and %)		185 (100,0%)				539 (100,0%)				134 (100,0%)				858 (100,0%)		<0,001	
Loss of smell and taste	Yes	34 (18,4%)	<0,001	56,70%	<0,001	20 (3,7%)	<0,001	33,30%	0,001	6 (4,5%)	<0,001	10,00%	0,239	60 (100,0%)	<0,001	<0,001	<0,001
	No	151 (81,6%)		18,90%		519 (96,3%)		65,00%		128 (95,5%)		16,00%		798 (100,0%)		<0,001	<0,001
Total (absolute number and %)		185 (100,0%)				539 (100,0%)				134 (100,0%)				858 (100,0%)		<0,001	
Diarrhea	Yes	39 (21,1%)	<0,001	28,70%	0,199	79 (14,7%)	<0,001	58,10%	0,587	18 (13,4%)	<0,001	13,20%	0,577	136 (100,0%)	<0,001	<0,001	<0,001
	No	146 (78,9%)		20,20%		460 (85,3%)		63,70%		116 (86,6%)		16,10%		722 (100,0%)		<0,001	0,001
Total (absolute number and %)		185 (100,0%)				539 (100,0%)				134 (100,0%)				858 (100,0%)		<0,001	
Contact with Covid-19 patient																	
Yes		116 (67,1%)	<0,001	21,30%	<0,001	351 (70,1%)	<0,001	64,50%	0,528	77 (59,2%)	0,035	14,20%	0,303	544 (100,0%)	<0,001	<0,001	<0,001
No		57 (32,9%)		21,90%		150 (29,9%)		57,70%		53 (40,8%)		20,40%		260 (100,0%)		<0,001	<0,001
Total (absolute number and %)		173 (100,0%)				501 (100,0%)				130 (100,0%)				804 (100,0%)		<0,001	
Disease prevalence																	
Diabetes		3 (7,7%)	<0,001	20,00%	0,365	8 (8,0%)	<0,001	53,30%	<0,001	4 (14,8%)	0,002	26,70%	0,093	15 (100,0%)	<0,001	0,247	<0,001

Bronchitis	9 (23,1%)	26,50%		19 (19,0%)	55,90%		6 (22,2%)	17,60%		34 (100,0%)		0,017	<0,001
Hypertension	20 (51,3%)	25,00%		48 (48,0%)	60,00%		12 (44,4%)	15,00%		80 (100,0%)		<0,001	<0,001
Asthma	4 (10,3%)	21,10%		12 (12,0%)	63,20%		3 (11,1%)	15,80%		19 (100,0%)		0,021	<0,001
Diabetes and hypertension	0 (0,0%)	0,00%		7 (7,0%)	87,50%		1 (3,7%)	12,50%		8 (100,0%)		0,034	<0,001
Bronchitis and hypertension	3 (7,7%)	33,30%		5 (5,0%)	55,60%		1 (3,7%)	11,10%		9 (100,0%)		0,264	<0,001
Others	0 (0,0%)	0,00%		1 (1,0%)	100,00%		0 (0,0%)	0,00%		1 (100,0%)		no result	no result
Total (absolute number and %)	39 (100,0%)			100 (100,0%)			27 (100,0%)			166 (100,0%)		<0,001	
Regular use of medication													
For Hypertension	20 (29,4%)	24,40%		50 (26,6%)	61,00%		12 (19,0%)	14,60%		82 (100,0%)		<0,001	<0,001
For diabetes	4 (5,9%)	20,00%		11 (5,9%)	55,00%		5 (7,9%)	25,00%		20 (100,0%)		0,116	<0,001
For heart	1 (1,5%)	14,30%		5 (2,7%)	71,40%		1 (1,6%)	14,30%		7 (100,0%)		0,102	<0,001
Others	43 (63,2%)	20,50%		122 (64,9%)	58,10%		45 (71,4%)	21,40%		210 (100,0%)		<0,001	<0,001
Total (absolute number and %)	68 (100,0%)			188 (100,0%)			63 (100,0%)			319 (100,0%)		<0,001	

* p-value in relation to the positive result in relation to the variable Symptoms and report yes (fever, breathing difficulty, loss of smell and taste, and diarrhea): 0.004; and in relation to percentage 0.007.

Table 3: Correlation between ELISA test positivity and other variables

	Hospital unit					
	Hospital 1		Hospital 2		Hospital 3	
Variables	p value	Significance	p value	Significance	p value	Significance
Gender	0,193	No	0,366	No	0,445	No
Profession area	0.8779	No	0.237	No	0,306	No
Work sector	0.7827	No	0.260	No	0.4513	No
If tests were performed previously	0.8507	No	0.112	No	0.1267	No
Type of test performed previously	0.8852	No	0,723	No	0.424	No
Test result performed	1,14E-02	Yes	0.016	Yes	0.000	Yes
Fever	0,589	No	0.870	No	0.03	Yes
Breathing difficulty	0.4758	No	0.126	No	0.4223	No
Loss of smell and taste	0	Yes	0.514	No	0	Yes
Diarrhea	0,08	No	0,899	No	0,51	No
Contact with Covid-19 patient	0,587	No	0,266	No	0,427	No
Comorbidities	0.9314	No	0.267	No	0.1487	No
Regular use of medication	0.5441	No	0.352	No	0.2233	No

Note: the relationship between the variables studied, using the chi-square or Fisher's exact tests, all considering a 5% significance level

Supplementary table 1A: Age of participants grouped into 10 years

	Hospital unit								
	Hospital 1		Hospital 2		Hospital 3		Total		
Age	Number (%)	p-value	Number (%)	p-value	Number (%)	p-value	Number (%)	p-value	p-value of the lines
18-27	49 (16,3%)		38 (16,4%)		32 (9,8%)		119 (13,9%)		0,154
28-37	101 (33,7%)		78 (33,6%)		111 (33,9%)		290 (33,8%)		0,052
38-47	89 (29,7%)		59 (25,4%)		111 (33,9%)		259 (30,2%)		<0,001
48-57	49 (16,3%)	<0,001	28 (12,1%)	<0,001	54 (16,5%)	<0,001	131 (15,3%)	<0,001	0,013
58-67	7 (2,3%)		8 (3,4%)		11 (3,4%)		26 (3,0%)		0,607
68-77	0 (0,0%)		2 (0,9%)		1 (0,3%)		3 (0,3%)		0,564
Uninformed	5 (1,7%)		19 (8,2%)		7 (2,1%)		31 (3,6%)		0,004
Total	300 (100,0%)		232 (100,0%)		327 (100,0%)		859 (100,0%)		<0,001

Supplementary Table 1B: Age of participants grouped at 10 years in relation to ELISA test results

	ELISA test															
	Positive				Negative				Undetermined				Total			
	Number (%)	p-value	% group	p-value	Number (%)	p-value	% group	p-value	Number (%)	p-value	% group	p-value	Number (%)	p-value	p-value of the lines	% p-value of the lines
Age																
18-27	25 (13,4%)		21,01%		79 (14,7%)		66,39%		15 (11,2%)		12,61%		119 (100%)		<0,001	<0,001
28-37	55 (29,6%)		19,03%		199 (37,0%)		68,86%		35 (26,1%)		12,11%		289 (100%)		<0,001	<0,001
38-47	60 (32,3%)	<0,001	23,17%	0,070	154 (28,6%)	<0,001	59,46%	<0,001	45 (33,6%)	<0,001	17,37%	0,363	259 (100%)	<0,001	<0,001	0,008
48-57	27 (14,5%)		20,61%		77 (14,3%)		58,78%		27 (20,1%)		20,61%		131 (100%)		<0,001	<0,001
58-67	10 (5,4%)		38,46%		10 (1,9%)		38,46%		6 (4,5%)		23,08%		26 (100%)		0,540	0,103
68-77	0 (0,0%)		0,00%		3 (0,6%)		100,00%		0 (0,0%)		0,00%		3 (100%)		no result	no result

Uninformed	9 (4,8%)	29,03%	16 (3,0%)	51,61%	6 (4,5%)	19,35%	31 (100%)	0,078	<0,001
Total	186 (100,0%)		538 (100,0%)		134 (100,0%)		858 (100%)	<0,001	

4 Discussion

Healthcare professionals and those that work in hospital settings are at high risk of exposure to SARS-CoV-2. Serological response assessments during the COVID-19 pandemic are crucial to understand the risk of infection. This study aimed to carry out a seroepidemiological survey of SARS-CoV-2 in workers from hospitals located in south of Minas Gerais, Brazil, in 2020. The work was carried out in a period prior to vaccination, which is critical for the transmission of the virus and during the quarantine, when people were advised to stay home and wear basic protection like masks and sanitizing alcohol.

The age and gender of the participants are important factors to understand the dynamics of the disease in population, because they are considered a risk factor for the disease, with a higher rate of deaths in patients over 50 years old in the first and second wave in 2020 in Italy [26]. The average age of the participants was 38 to 43 years, with the highest positivity rate between 38 and 47 years old with 32.6% (supplementary table 1A), a similar perspective was found in Minas Gerais population infected by the virus, which was between 30 and 39 years old, with 23.9% of cases in the state until December 2020 [27]. The stratification by age performed with an interval of 10 years (supplementary tables 1A and 1B) follows what was carried out in Ireland that evaluated hospital professionals [25].

In a study carried out with hospital workers in Denmark, the average age was 44.4 years, with positivity in antibody tests of 13.5% under this average [28].

Most of the participants are health professionals (74.5%). 17.5% of the professionals were in direct care of patients with Covid-19, while 58% of them did not work directly with these patients (Table 1). However, when analyzing only the sample of health professionals (640), the participants who had direct contact with patients with Covid-19 was 23.1% and without direct contact with patients with Covid-19 was 76.8%.

The present study showed a positivity of 21.6% in the seroprevalence of SARS-CoV-2 in the three researched hospitals. The field hospital (hospital 3) and Santa Casa de Misericórdia de Alfenas (hospital 1) worked specifically with the treatment of patients with Covid-19, with positivity rates of 22.9% and 20.3% respectively. These two hospitals serve several cities in their region because they are regional health centers. Hospital 2, a private hospital, had a positivity rate of 21.6% (Table 1). In a study carried out in two hospitals in Germany with their professionals, between July and September 2020, it revealed a positivity percentage of 38.5% in the first hospital and 61.5% in the second hospital, using IgG antibody tests. However, in relation to the total sample, the positivity in the tests was 1.4% [29]. In another study carried out in Denmark, health professionals in direct contact with Covid-19 patients from different hospitals who were invited to participate voluntarily, in April 2020, they presented a positive result for antibody tests, 2.67% presented a result for IgG tests, 2.81% developed IgM antibodies and 4.04% had IgG, IgM or both [28]. A study carried out in a university hospital in Ireland, between May and June 2020, showed a positivity of 15.5% in IgG antibody tests [25]. In the study carried out in Milan, between February and May 2020, in a university hospital, with samples collected at three different times, the initial phase of the pandemic, 1 and 2 months, with a progressive increase in positive tests at each time of the test performed, 0.4%, 4.2% and 4.6% respectively [30]. In turn, in a hospital in Belgium between May and June 2020, the positivity rate in health professionals was 7.4% [31]. It is observed that the positivity rates in the tests in each hospital, in this study, were higher than in some studies carried during previous periods of time and lower in similar periods.

When analyzing the percentage of positive cases notified by the municipal health departments, in relation to the total population of municipality, where the ELISA tests were carried out, in the year 2020 with the bulletin of December 30th, in Alfenas they were 3.3% (2,429/73,774), in Poços de Caldas 1.7% (2,625/152,435) and in Varginha 1.7% (2,174/123,081) [27]. Prakash *et al.* [32], evaluating seropositivity for IgG antibodies against SARS-CoV-2 in the city of Ahmedabad, India, found 17.61% seropositivity and no statistically significant difference for both sexes. These data may suggest underreporting of positive cases in the cities where the tests were performed.

The total number of participants in terms of gender was 71.4% women and 28.6% men (Table 1), however, seropositivity in women was 22.8% and in men 18.7% (table 2). This data is similar to the data found in the study by Hildebrandt *et al.* [29], with a greater number of positives in women: 76.9% women and 23.1% men. Data from the epidemiological bulletin of the State of Minas Gerais with cumulative frequency up to December 30, 2020, shows a higher rate of female positivity with 51% [27], these data are based on the general population of the state, and according to this result, in the ELISA tests the percentage of positivity was 75.3% of women and 24.7% of

men.

Health professionals had 20.9% of positive results (Table 2). This demonstrates how these professionals were exposed to the virus in their workdays. Venugopal *et al.* [33] assessed seroprevalence among healthcare workers in a New York City hospital and found 27% positivity for the SARS-CoV-2 antibody. Bryan *et al.* [34] reported 29% positive cases for the presence of SARS-CoV-2 antibodies also in New York City. Gomez-Ochoa *et al.* [35] showed that seropositivity in health professionals was 7%.

When classifying professionals by work sector, it was observed that professionals in direct contact with patients had 22.0% positivity, while professionals in indirect contact had 21.5% positivity (Table 2). Gomez-Ochoa *et al.* [35] showed that 43% of professionals with a positive result worked in hospital wards and non-emergency sectors during patient triage. In turn, Prakash *et al.* [32], showed a significantly lower seropositivity (13.64%) for health professionals compared to non-health professionals (18.71%). The work by Purswani *et al.* [36] showed that one-third of hospital healthcare workers were seropositive for SARS-CoV-2 by the end of the first wave in New York. Seroprevalence differs by job function and workplace, with the highest estimated risk for nurses and the emergency department, respectively. In turn, professionals in the administrative, laboratory and imaging areas had a high positivity rate (22.6% and 30.3% respectively) (Table 2). Brousseau *et al.* [37] show 11.7% positive serology for SARS-CoV-2 among healthcare workers in Quebec, Canada. Of these, 71.0% had been previously diagnosed with Covid-19. Seroprevalence varied between hospitals, from 2.4% to 3.7% in low-incidence regions and from 17.9% to 32.0% in hospitals with outbreaks involving 5 or more health workers. The highest seroprevalence was associated with working in a hospital where they occurred, being a nurse or nursing assistant, or an orderly and black or Hispanic ethnicity. Lower seroprevalence was associated with work in the intensive care unit or emergency department.

The results of contamination in hospital environments may be related to several factors, such as the incorrect use of PPE, the non-use of such equipment or PPE, or the use of less efficient PPE. In relation to administrative professionals, since they have contact with the public that arrives at hospitals, both asymptomatic and symptomatic. In this sense, the work by Gómez-Ochoa *et al.* [35] presented 4.7% positivity in professionals without proper use of PPE. In this way, the proper use of PPE and social distancing measures are of fundamental importance to reduce the risk of contamination by SARS-CoV-2 [38].

66.5% of the participants reported having performed tests for Covid-19 and of these, 5.4% with positive results in comparison to the total number of participants (Table 1). However, when performing the percentage considering the total number of reports of previous tests, the percentage is 8.0% (46/571). The comparison between the positivity of the ELISA tests with the results of the tests previously performed by the participants showed that 22.3% (118/530) had a positive result in the ELISA tests (table 2), suggesting a concordance between our tests and the tests previously performed. Factors such as the time of sample collection can directly impact the test result [16]. In this sense, the ELISA test was adequate to assess the prevalence of antibodies against the virus [16,39].

Another important variable was to assess whether the participants had contacts with symptomatic people for the disease [38]. 63.3% of the participants reported direct contact with patients with Covid-19, with the highest rate presented by hospital 1 (74.0%), hospital 2 (65.5%) and hospital 3 (52.0%) and 6.4% do not know or did not answer (Table 1). Our results showed that 21.3% of the participants who reported having contact with sick people had a positive result in the ELISA tests (Table 2), which may be associated with the observed seropositivity rate. Prakash *et al.* [40] found a seroprevalence of 31.92% considering people who had contact with Covid-19 cases in the city of Ahmedabad, India.

Understanding how the disease behaves in the participants is important to analyze whether the symptoms presented are common, which are the most recurrent and, therefore, be able to seek medical assistance when these symptoms develop. Among the symptoms most reported by the participants, diarrhea is the most common with 15.8% and 28.7% of positivity in the ELISA tests, the percentage of positivity on loss of smell and taste with 7% of reports and 56.7% of positivity, fever with 6.4% of reports and 34.5% of positivity and respiratory difficulty with 5.8% of reports and 34.0% of positivity (Tables 1 and 2 respectively). The symptom of diarrhea, in addition to Covid-19, can have different etiologies, however it is one of the most frequent symptoms reported by patients, with about 10.4% of reports [41]. The work by Mair *et al.* [42] carried out with data from hospitalized patients showed that 69% had fever, 38% loss of smell, 29% loss of taste, 9% diarrhea and 10 to 20% respiratory difficulty. The other evaluated the presence of 11 symptoms common to the disease, 63.5% had three or more symptoms, 56.5% had changes in smell and taste, 52.1% fever, 25.6% diarrhea, 23.1 difficulty breathing [43].

Another important discussion is whether the participants have a prevalence of diseases and the use of

drugs to control them, which are directly related to the development of a serious Covid-19 outcome [10,44]. 19.2% (166/859) of the participants (Table 1) had some type of morbidity and their positivity in the ELISA tests was 25.0% hypertension, 26.5% bronchitis, 21.1% asthma, 20 .0% diabetes, 33.3% bronchitis and hypertension (Table 2).

12.6% (109/859) of the participants were taking medication related to the comorbidities mentioned above (Table 1), and among these, in relation to the ELISA tests, the use of hypertensive drugs (29.4%) for diabetes was positive (5.9%) and for the heart (1.5%) (Table 2). In addition, it is observed that most participants who reported using some medication not related to the disease as a risk factor for Covid-19 (24.4%) had a positive result in the ELISA tests of 63.2% (table 1 and 2) that is, there were more people who took medication (312 participants, 19.2%) than those who reported having some type of disease (166 participants, 37.0%).

The association between ELISA test positivity and the studied variables indicated a statistically significant association for previous ELISA test results for Covid-19 in the three hospitals, loss of smell and taste in hospitals 1 and 2, and fever in hospital 3 (table 3). This result highlights the relevance of the data found.

Thus, in the population studied, the highest exposure of health professionals with the lowest exposure to professionals from other areas with patients with Covid-19 did not show a significant difference in prevalence between the groups. However, there was a significant difference in relation to reported symptoms associated with positive ELISA test results and with contact with a Covid-19 patient.

5 Conclusion

It is concluded that the results of the ELISA tests to detect antibodies to SARS-CoV-2 in hospital workers showed that there was no significant difference between workers from different areas of profession, even with greater or lesser exposure during their profession. However, it demonstrated that all hospital professionals can be exposed to infection.

The results determine that there was a significant difference between the reported symptoms and the positive result of the ELISA tests, as well as the contact with a patient with Covid-19.

Among the variables studied, those that correlated significantly with positive serology for Covid-19 were past symptoms of fever and anosmia (loss of taste) and ageusia (loss of smell). So far, no similar work has been carried out in the region.

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The authors declare that the present study had no conflict of interest.

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3 CONSIDERAÇÕES FINAIS

Conclui-se que os resultados dos testes ELISA para detectar anticorpos para *SARS-CoV-2* nos trabalhadores hospitalares demonstraram que não houve diferença significativa entre os trabalhadores de áreas de profissão diferentes, mesmo que com maior ou menor exposição durante a realização de sua profissão. Entretanto, demonstrou que todos os profissionais hospitalares podem estar expostos à infecção.

Os resultados determinam que houve diferença significativa entre os sintomas relatados e resultado positivo dos testes ELISA, assim como o contato com paciente com Covid-19.

Dentre as variáveis estudadas, aquelas que se correlacionaram significativamente à sorologia positiva para Covid-19 foram sintomas pregressos de febre e anosmia (perda de paladar) e ageusia (perda de olfato). Até o momento, não há trabalhos similares realizados na região.

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ANEXO A – Folha de rosto da submissão do artigo.

Brazilian Journal of Microbiology

Seroprevalence of SARS-CoV-2 in hospitals workers in the south region of Minas Gerais state, Brazil: An analysis in the pre-vaccine period

--Manuscript Draft--

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Abstract:	The SARS-CoV-2 infection seroprevalence can be performed by detecting anti-SARS-CoV-2 antibodies. The survey is essential to understand the disease transmission dynamic in the studied population. This study aimed to carry out a seroepidemiological survey of SARS-CoV-2 in three hospitals located in the south of Minas Gerais state, Brazil. Samples were collected from August to December 2020. SARS-CoV-2 vaccines were still not available. Enzyme-linked immunosorbent assays (ELISA) were performed on sera from 859 participants. Results: The mean age was 38 years old. Most of the participants were women (71.4%). The majority of the participants were classified as health care professionals with direct or indirect contact with Covid-19 patients (74.5%). Considering clinical symptoms, 15.8% reported diarrhea, 6.4% fever, 5.8% breathing trouble, and 7.0% loss of smell and taste. Many participants reported contact with infected patients (63.35%). Regarding ELISA tests, 21.6% of the participants presented positive results. Hospital 3 had the highest positivity (8.7%), followed by hospital 1 (7.1%) and hospital 2 (5.8%). The prevalence was higher in women compared to men (16.3% and 5.4% respectively). The highest positivity (17.3%) was observed among healthcare professionals. However, professionals who worked exclusively with Covid-19 showed lower positivity when compared to professionals from other sectors (4.3% and 14.0% respectively). An association was detected between positivity in the ELISA test and previous serological tests, patients who had previous contact with Covid 19 and the presence of fever, loss of smell, and taste. Conclusion: clinical symptoms in association with serological tests are important tools for monitoring the disease among health professionals.
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