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Evaluation of Symptoms and Viral Load in the Population of the Institute of Biology of the Federal Fluminense University During the COVID-19 pandemic

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ABSTRACT

The SARS-CoV-2 infection is highly transmissible, pathogenic and has such as main detection technique the quantitative reverse transcription polymerase chain reaction (qRT-PCR). Since its discovery in 2019, countless questions about parameters that could favor SARS-CoV-2 infection were raised. Therefore, we analyzed in this study nasopharyngeal swabs of part of the population floating at the Institute of Biology of the University Federal Fluminense, Niteroi, Brazil, between August and September 2021 correlating the data to age, race, gender, demographic distribution and comorbidities pre/post SARS-CoV-2 infection. We showed that individuals with high number of viral copies are more susceptible to developing headache, runny nose, sneezing, fever, pain when swallowing and cough. Additionally, systemic arterial hypertension was not a comorbidity associated to SARS-CoV-2 infection. Regarding age, individuals aged 60 or over presented higher number of positive cases and according with self-declared data about gender, race and demographic distribution, female white and brown residents in northern region of Niteroi are more susceptible to SARS-CoV-2 infection. Thus, we conclude that population more vulnerable aged 60 or over and female gender are more susceptible the SARS-CoV-2 infection.

Keywords: SARS-CoV-2; COVID-19; qRT-PCR; symptomatology; Niterói

INTRODUCTION

Since its discovery in Wuhan, China, in December 2019, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has resulted in numerous cases of mortality and morbidity. According with World Health Organization (WHO), in September 2022, more than 600 million cases were confirmed globally with 6 million deaths approximately [1]. In Brazil, the first case of SARS-CoV-2 was registered in February 2020 being the infection spreading across the country between March and April 2020 [2] and in 2022 registering more than 34 million cases with 683 thousand deaths [1], putting country the epicenter of the COVID-19 epidemic in the Americas. Analyses the spatial progression in Brazil revealed that São Paulo and Rio de Janeiro states were the main dissemination centers of SARS-CoV-2, including the VoCs variants (variants of concern) [3]. Additionally, Rio de Janeiro was also the background to the emergence and worldwide spread of the Variants of Interest (VoI) Zeta (P.2) [4]. Niteroi, a city of state of Rio de Janeiro, notified the first case on 12th March 2020, and the next month, seventeen fatal cases associated to the SARS-CoV-2 infection were reported with lethality rate of 14.29% [5]. A study retrospective genomic in blood donors of the Rio de Janeiro showed that 4.0% were positive for SARS-Cov-2, with weighted prevalence of 3.8% and highlight for parameters such as youth, sociodemographic and educational standard low [6]. On the other hand, it was showed that state residents of Rio de Janeiro aged 60 or older are more susceptible to infection followed by death approximately 11-13 days post-infection. Additionally, parameters such as high people flow, high social and economic inequality was also used to available the lethality tax in administrative region of the Rio de Janeiro state (Coastal lowlands, South-central Fluminense, Green coast, Mid-Paraíba, Metropolitan, Northwest Fluminense, North Fluminense and Mountainous) with higher rates in the metropolitan region [7]. The SARS-CoV-2 affects predominantly the respiratory system and produces symptons ranging from upper respiratory distress to severe pneumonia [8] however, complication in other systems including cardiovascular, neurological and renal also may occurs and contributes to the death [9]. Pre-existing comorbid illnesses such as hypertension, diabetes, obesity, cardiovascular diseases (CVDs), chronic kidney diseases (CKDs), and chronic obstructive pulmonary disease (COPD) are linked to major susceptibility and severity of infection [10]. On the other hand, independent of hospitalization or not during SARS-CoV-2 infection symptoms such as fatigue, pain and memory loss are the symptons most prevalent 2 years after SARS-CoV-2 infection [11]. Several tests for COVID-19 diagnosis include Nucleic Acid Amplification Testing (NAAT) such as RT-PCR, Computed Tomography scan (CT-scan), Protein testing via ELISA, Point of Care Tests (POCT) such as side flow assays, use of biosensors [12]. However, quantitative reverse transcription polymerase chain reaction (qRT-PCR) is considered the gold standard technique for detection of SARS-CoV-2 patients [13] from analyzes of nasopharyngeal swab or mask swab [14]. Therefore, the goal of this study was to identify individuals positive for SARS-CoV-2 frequenters at Institute of Biology of the University Fluminense Federal, Niteroi, Rio de Janeiro and group them according to symptomatology, race, age, gender, demographic distribution and pre-existing comorbidities.

MATERIAL AND METHODS

Data Source

Using a retrospective cohort study of part of the population housed at the Institute of Biology of the University Federal Fluminense, Niteroi, Brazil, between August and September 2021, 2586 nasopharyngeal swabs samples were collected, being only 158 randomly selected. The period chosen was due to the high demand for tests. The volunteers consent for the release of health records was necessary due to approval by the ethics committee CAAE with registration number 30623520500005243. Different groups were separated according to variables ethnicity, age, gender, demographic distribution, symptoms and comorbidities acquired pre infection.

Detection and quantification of SARS-CoV-2 by RT-qPCR

RNA extraction from the samples was performed using The ReliaPrep™ Viral TNA Miniprep (PROMEGA, Inc. Madyson, USA System), according with manufacturer's instructions. Once time extracted RNA, the samples were amplified and genetic material detected by qRT-PCR technology (Allplex™) SARS-CoV-2/FluA/FluB/RSV (ASFR, Seegene Technologies Inc; Seul, Coreia do Sul) at Applied Biosystems 7500 Real-Time PCR, through commercial kit molecular SARS-CoV-2 (E/RP). ASFR is a qRT-PCR multiplex assay that targets the N, RdRP, and S genes of SARS-CoV-2, influenza A, influenza B, and respiratory syncytial virus (RSV) through duplex assay of reverse transcription, amplification, detection, and differentiation of the genetic material (viral RNA), however, it was also applied for the diagnosis and epidemiological surveillance of SARS-CoV-2. Briefly, 15 µL of each sample were diluted in 45 µL of RNase-free water and added in qPCR/96-well plate, being 5 µL transferred to another plate pre-treated with 16 µL of qPCR master mix - 5 µL of MOM (MuDT Oligo Mixture, containing dNTPs, oligos, primers and TaqMan 5' fluorophore / 3' Black Hole Quencher probes), 5 µL of enzymes, 5 µL of RNase-free water and 1 µL of internal control for each reaction. The sequences of primers and probes used were from Corman et al. [15] as shown in (Table 1). The process of target-specific amplification with fluorescently probes was used to determine the presence of SARS-CoV-2 and RNAse P. All samples extracted were packaged and stored according to the OMS guidelines. The data were expressed by number of viral genetic replication cycles (Cq), being the samples with Cq < 25 classified as high viral load, Cq between 25-30 as moderate viral load, and Cq > 30 as low viral load. After, RNA from the samples was extracted using ReliaPrep™ Viral TNA Miniprep (PROMEGA, Inc., Madyson, USA) System, according with the manufacturer's instructions. The total volume obtained for each sample were 200 µL.

Table 1: Primers sequences and probes from E protein used in real-time RT-qPCR for SARS-CoV-2 detection

Gene	Oligonucleotides	Sequence
RdRp	RdRp_SARSr-F	GTGARATGGTCATGTGTGGCGG
RdRp	RdRp_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ
RdRp	RdRP_SARSr-P1	FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ
RdRP	RdRp_SARSr-R	CARATGTTAAASACACTATTAGCATA
E	E_Sarbeco_F	ACAGGTACGTTAATAGTTAATAGCGT
E	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ
E	E_Sarbeco_R	ATATTGCAGCAGTACGCACACA

Statistical analysis

The data were exposed in table format with the groups separated in symptomatic patients and positive PCR for SARS-CoV-2 (control group), asymptomatic patients and positive for SARS-CoV-2, asymptomatic patients and

negative for SARS-CoV-2, and patients only exposed and negative PCR for SARS-CoV-2. Prism 8.0 software (GraphPad Inc., CA, USA) was used for statistical analysis. The statistical analyses included Student's t-test and One-way ANOVA, followed by the Bonferroni test. The data are expressed as the means ± standard deviation of at least three independent experiments.

RESULTS

Collected samples of floating volunteers by the Institute of Biology of the University Federal Fluminense were analyzed, 158 samples randomly selected being 57 samples positive, and 101 negatives for SARS-CoV-2. During the samples collect, volunteers were asked about clinical manifestations, comorbidities diagnosed in the past, age, demographic distribution, gender, and race being all the information assigned to their respective samples. The first step was to list the clinical manifestations in both positive and negative groups for SARS-CoV-2. The comparison between positive/negative cases and symptoms not present significant statistical difference, except for fever (Table 2). The main symptoms reported by volunteers positive for SARS-CoV-2 were headache, runny nose, pain when swallowing, and cough. However, volunteers negative for SARS-CoV-2 also reported headache, runny nose, diarrhea, fever, pain when swallowing, and cough, showing symptomatological nonspecificity. The viral load is a parameter to evaluate the genome size and its analyses is proportional inversely to the number of cycles. Therefore, our analyzes showed that volunteers with a higher level of infection are more susceptible to developing headaches, runny nose, sneezing, fever, pain when swallowing, and cough. However, although reported by only 1 or 2 volunteers, body pain and chills were also considered as clinical manifestations of individuals with a high level of infection (17.4 and 19.9 of viral load). The comparison between viral load and symptoms showed no statistical significance, except for cough (Table 3). The pre-existing comorbidities also were listed to each group (SARS-CoV-2 negative and positive) (Table 4). Systemic arterial hypertension was more prevalent in the SARS-CoV-2-positive and negative groups, which suggests that there is no directly proportional relationship between comorbidity and SARS-CoV-2 infection. On the other hand, a greater number of SARS-CoV-2 negative volunteers reported having diabetes and chronic kidney disease pre-existing. The comparison between positive/negative cases and comorbidity showed no statistical significance. To understand the distribution and prevalence of the infection among volunteers, we separated the samples according with age, sex, race, and self-declared demographic distribution (Table 5). Volunteers aged 60 or over were more susceptible to the infection. When we analyzed the relationship between gender, we observed that there is a prevalence in the female gender. An interesting fact was when we separated the results by race (white, brown, and black). Our analyses show that white and brown volunteers are more likely to be infected by SARS-CoV-2 compared to the black volunteers. For last, we grouped the volunteers according to demographic distribution and observed that most cases are concentrated in the northern region of Niteroi (Fonseca and Pendotiba) when compared to the central and beach regions.

Table 2: Relationship between samples from volunteers and clinical manifestations

	Positive (n=57)				Negative (n=101)			
	Female		Male		Female		Male	
Symptoms	n	%	n	%	n	%	n	%
Loss of taste	53	93	4	7	98	97	3	3
Loss of taste	57	100	0	0	100	99	1	1
Loss of smell	52	91.2	5	8.8	99	98	2	2
Chills	45	98.2	0	0	101	100	1	1.8
Headache	51	78.9	23	22.8	78	77.2	12	21.1
Nasal congestion	24	89.5	3	3	98	97	6	10.5
Runny nose	56	42.1	52	51.5	49	48.5	33	57.9
Diarrhoea	51	98.2	1	1.8	93	92.1	52	51.5
Shortness breath	56	89.5	9	8.9	92	91.1	6	10.5
Body pain	56	98.2	4	4	97	96	1	1.8
Eye pain	57	100	1	1	100	99	0	0
Sneezing	57	100	0	0	97	96	4	4
Fatigue	56	98.2	9	8.9	92	91.1	1	1.8
Fatigue	35	61.14	7	6.9	94	93.1	22	38.6
Muscle pain	51	89.5	9	8.9	92	91.1	6	10.5
Pain when swallowing	33	57.9	36	36.5	65	64.4	24	42.1
Cough	24	42.1	49	48.5	52	51.5	33	57.9

[a] n represents the number of responses reporting the presence of any symptom after suspicion of the disease. [b%] represents the proportion of all responses. [c] the p-values using the Mann-Whitney test.

Table 3: Relationship between symptoms and viral load

	Positive (n=57)				
Symptoms and viral load	Yes	Viral load	No	Viral load	P Value
Loss of taste	4	26.1	53	26.3	0.815
Loss of smell	5	27.7	52	26.1	0.989
Chills	1	19.9	56	26.1	0.648
Headache	12	25.1	45	26.6	0.75
Nasal congestion	6	29.5	51	25.9	0.346
Runny nose	33	24.4	24	28.8	0.074
Diarrhoea	1	36.6	56	21.1	0.191
Shortness breath	6	28.7	51	26	0.304
Body pain	1	17.4	56	26.4	0.287
Sneezing	33	24.4	24	28.8	.....
Fatigue	1	36.9	56	26.1	0.153
Fever	22	35	27	94	0.399
Muscle pain	6	28	51	26.1	0.559
Pain when swallowing	24	25.1	33	27.1	0.39
Cough	33	23.3	24	30.4	<0.001

Table 4: Relationship between comorbidities and positive and negative cases

	Positive (n=57)				Negative (n=101)			
	Female		Male		Female		Male	
Comorbidities	n	%	n	%	n	%	n	%
HAS	51	89.5	9	10.5	86	85.5	15	14.9
Pre-diabetes	57	100	0	0	100	99	1	1
Diabetes	56	96.2	0	0	97	96	4	4
Chronic cardiac disease	56	96.2	1	1.8	98	97	3	3
Fibromyalgia	57	100	0	0	100	99	1	1

Crohn's disease	57	100	1	1	100	99	1	1
Alzheimer's disease	57	100	0	0	100	99	1	1
Sinusitis	57	100	0	0	100	99	1	1

DISCUSSION

The qRT-PCR method is regarded as the gold standard diagnosing the SARS-CoV-2 virus, it is the recommended diagnostic test for symptomatic and asymptomatic patients 13. This technique is more commonly used due to its sensitivity and specificity higher, which enables rapid screening of a large number of specimens within a short time. Viral genome sequencing takes a longer time at a higher expense than qRT-PCR assays and makes it unsuitable for diagnosis and patient screening [16]. Therefore, we used qRT-PCR to quantify the number of viral copies of samples obtained from volunteers attending the Institute of Biology of the University Federal Fluminense, Niteroi, Brazil. The results showed that people independent of the races, age, gender, and demographic distribution can be infected by SARS-CoV-2. However, people white and brown races, female gender, and aged 60 years or older are most susceptible to the infection. Symptoms such as headache, runny nose, sneezing, fever, pain when swallowing, and cough are the most frequent during the infection. Curiously, pre-existing comorbidity, mostly systemic arterial hypertension, do not have a direct relationship or were not a predisposing factor for susceptibility to SARS-CoV-2 infection. SARS-CoV-2 infection affect countless people, leaving sequelae that can remain for a long time 1. A study with hospitalized patients in Wuhan, China, showed that symptoms such as fever, fatigue and dry cough are the most presented by infected patients while sputum production, headache, haemoptysis, diarrhoea, anorexia, sore throat, chest pain, chills and nausea and vomiting are the less manifested [17, 18, 19]. However, a study carried out with brazilian patients showed that fever, fatigue, headache, myalgia, chills, changes in taste or smell, and cough are more frequent during infection 20 corroborating our findings. Based on comparisons between countries of different continents, it is interesting to highlight that independent of the circulating strain in the country, genetic factors influence directly the host immune response and symptom intensity. Additionally, according with Gouvea-Reis and coworkers [20], pre-existing comorbidities such as hypertension and diabetes, together with advanced age are risk factors that favor infection by SARS-CoV-2. On the other hand, Palamim and coworkers [21] in their analysis with hospitalized brazilian patients showed that cardiopathies, diabetes mellitus and obesity were pre-existing comorbidities that most favored SARS-CoV-2 infection in patients. Curiously, our data did not indicate systemic arterial hypertension as a predisposing factor to infection. The age has been a factor that directly influences in the susceptibility to infection. Some studies with SARS-CoV-2 have shown that older individuals, mainly aged 60 or more are those most diagnosed with infection [20, 22, 7]. Also, Ghanchi and coworkers [23] showed data that individuals aged 80 or more had 2.5 times higher odds of testing positive than those aged 0-10 years. The most susceptibility of these individuals can be explained by smaller early interferon (IFN) response to the infection compared to the strong early interferon response seen in younger patients. IFN is the first defense line innate immune system against viral infections, being activated after recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs), resulting in the triggering cell signaling cascades and production of interferon by the cell [24]. Therefore, an early viral response impairs: complete viral replication, the increase subgenomic RNA, and the number of infectious viruses [25]. Characteristics such as race and gender have also attracted attention for the rate of positive cases. A study with brazilian adults recently published that non-white individuals are more seroprevalence to the SARS-CoV-2 infection, especially those of

mixed race and/or indigenous descent compared with whites [26]. Curiously, the mortality rates between blacks and mixed individuals are 81% and 45% higher than whites, respectively with disparities more pronounced in the young/adult population [27]. However, it was seen by Palamim and coworkers [21] that white people, males gender and aged between 25 and 60 years are most susceptible to the infection. When we analyzed the risk of death, the profiles were different. Male gender, aged 61 or older, brown, black, and indigenous race would have an increased chance of death. If these profiles were added to comorbidities such as hepatic disorder, immunosuppressive disorder, and kidney disorder, the chances would be considerably increased [21]. In contrast, our data diverge from the literature by showing that females and/or white people are more susceptible to SARS-CoV-2. Regions far from the central and southern areas of cities constantly suffer from poor infrastructure. The Niteroi city, a metropolitan region of Rio de Janeiro, lacks these same problems in neighborhoods located in the northern zone. According to our data, individuals from the northern region were more prevalent in the SARS-CoV-2 positive group compared to individuals living in the central and beach regions. A plausible explanation for higher comorbidity can be attributed to lower schooling and income and higher number of individuals in the household. The relationship between more vulnerable = less health is not a new phenomenon, and SARS-CoV-2 pandemic exacerbated existing structural inequalities [27]. Also, the social conditions to which minority populations are exposed, predisposes it to higher rates of infection and opportunistic infections [28]. Herein, we showed some factors that may favor the acquisition of SARS-CoV-2 as well as a possible direction of the spread of SARS-CoV-2 through the Niteroi city. However, our study has some limitations, such as the absence of genetic variants associated with a SARS-CoV-2 infection, missingness in vaccination data, and whether it was a recurrent infection or not, reducing analyses and comparisons within the study.

CONCLUSION

In this study, we analyzed nasopharyngeal swabs of part of the population floating at the Institute of Biology of the University Federal Fluminense, Niteroi, Brazil, between August and September 2021 correlating the data to age, race, gender, demographic distribution and comorbidities pre/post SARS-CoV-2 infection. We observed that people white and brown races, gender, and aged 60 years or older are most susceptible to the SARS-CoV-2 infection. Headache, runny nose, sneezing, fever, pain when swallowing, and cough were the symptoms more most present in positive cases. Additionally, pre-existing comorbidities did not influence susceptibility to infection. Social classes most vulnerable showed higher infection rate.

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