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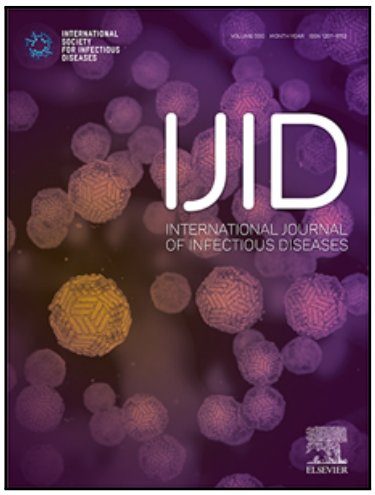

SARS-CoV-2 variant of concern P.1 (Gamma) infection in young and middle-aged patients admitted to the intensive care units of a single hospital in Salvador, northeast Brazil, February 2021

Carolina Kymie Vasques Nonaka , Tiago Gräf , Camila Araújo de Lorenzo Barcia , Vanessa Ferreira Costa , Janderson Lopes de Oliveira , Rogério da Hora Passos , Iasmin Nogueira Bastos , Maria Clara Brito de Santana , Ian Marinho Santos , Karoline Almeida Felix de Sousa , Thamires Gomes Lopes Weber , Isadora Cristina de Siqueira , Clarissa Araújo Gurgel Rocha , Ana Verena Almeida Mendes , Bruno Solano de Freitas Souza

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SARS-CoV-2 variant of concern P.1 (Gamma) infection in young and middle-aged patients admitted to the intensive care units of a single hospital in Salvador, northeast Brazil, February 2021

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Highlights

- A second COVID-19 wave accelerated after the emergence of the P.1 lineage (Gamma).
- An increased rate of younger patients needed intensive care during the second wave.
- More COVID-19 patients without comorbidities entered the ICU in February 2021.
- A high prevalence of Gamma was found in younger ICU patients in February 2021.

Abstract

Objectives: To evaluate changes in COVID-19 patients' characteristics occurring after the emergence of the SARS-CoV-2 variant of concern (VOC) P.1 (Gamma) by comparing the clinical, demographic and laboratory profile of patients hospitalized during the first (May-July 2020) and second (December 2020 - February 2021) pandemic waves.

Methods: Data was collected from records of COVID-19 patients (n=4164) admitted to a single hospital in Salvador, Northeast Brazil. SARS-CoV-2 genome sequencing was performed in nasopharyngeal swab samples from 12 patients with age < 60 years-old admitted to the intensive care units (ICU) in February 2021.

Results: From June 2020 to February 2021, the median age of patients admitted in the ICU decreased from 66 to 58 years ($p<0.05$), accompanied by an increased proportion of patients without comorbidities (15.32% vs 32.20%, $p<0.0001$). A significant reduction in the Ct values of SARS-CoV-2 RT-PCR tests was observed in the second wave ($p<0.0001$). Sequencing analysis detected lineage Gamma in all 12 ICU patients sampled in February 2021.

Conclusion: Our results demonstrated an increased proportion of younger adults without comorbidities with severe disease during the second COVID-19 wave, shortly after the confirmation of local Gamma circulation.

Keywords: COVID-19; SARS-CoV-2; variants of concern; P.1; Gamma.

Introduction

New SARS-CoV-2 variants have shown concern regarding their increased infectivity and possible immune escape[1]. According to the World Health Organization (WHO), there are currently four variants of concern (VOCs), all with key mutations in the receptor binding domain (RBD) of the spike protein[2]. These are Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1) and Delta (B.1.617) lineages.

In Brazil, two SARS-CoV-2 lineages - B.1.1.28 and B.1.1.33 - were predominant during the first surge of infections in the first semester of 2020 [3,4]. The emerging Brazilian variant Gamma, derived from B.1.1.28 lineage, began spreading rapidly later in 2020. Initially detected in Amazonas state, Gamma was responsible for a public health calamity in this state[5,6]. Currently, Gamma is being detected at increasing rates from January 2021 on throughout Brazil, becoming the predominant lineage associated with the second wave of infections (http://www.genomahcov.fiocruz.br). Following an acceleration of the transmission rates in 2021, Brazil has become the epicenter of the COVID-19 pandemic, with over 13 million confirmed cases and 350,000 deaths (https://www.covid.saude.gov.br).

Gamma has 21 lineage-defining mutations, including ten in the spike protein, three of them in the RBD (K417T, E484K and N501Y), showing a surprising convergence with the B.1.351 RBD. These three mutations in the RBD combined were shown to increase the receptor binding affinity[7]. The mutations found in Gamma were associated with increased transmissibility[6], higher viral load[5] and propensity for immune evasion[8] and SARS-CoV-2 reinfection[9].

Along with the dissemination of Gamma, there were also reports of an increased percentage of young patients evolving with severe disease[8]. However, many other questions remain unanswered, including possible increased fatality rate, disease severity in people without known SARS-CoV-2 risk comorbidities, reduced time of evolution from the first symptoms, and hospitalization.

Recently, public health authorities in the state of Bahia (Northeast Brazil) have confirmed the circulation of Gamma in the state. The first reports of Gamma detection in Salvador date from late December 2020 and early January 2021, and they are linked to individuals with travel history to Manaus[10]. This was followed by a rampant increase in the number of hospitalizations and deaths due to COVID-19 in Bahia in February and March 2021 (https://bi.saude.ba.gov.br/transparencia/). However, the clinical and demographic features of this epidemic second wave, as well as the association with SARS-CoV-2 lineages are still up to be characterized. Herein, we report changes in the profile of patients admitted in the intensive care unit, due to COVID-19, in a private hospital in Salvador, Bahia capital city, in February 2021, with possible involvement of the local circulation of the Gamma variant of concern.

Methodology

Study design and procedures

A cross-sectional study was performed at the São Rafael Hospital, a reference private general hospital in Salvador, Bahia, Northeast Brazil. Two time periods with increased numbers of hospital admissions were selected for data analysis, corresponding to the first wave (May-July 2020) and second wave (December 2020-February 2021) of COVID-19 hospital admissions.

Data regarding the number of confirmed COVID-19 cases admitted at the hospital, along with their clinical and demographic characteristics was obtained from the hospital health information System and electronic medical records (n=4164). Data regarding confirmed COVID-19 cases in the city of Salvador and the state of Bahia was obtained from https://bi.saude.ba.gov.br.

The nasopharyngeal swabs were obtained and tested by multiplex real-time PCR Allplex™ SARS-CoV-2 assay (Seegene Inc, Seoul, Korea) in the laboratory of São Rafael Hospital, as part of routine diagnostic procedures. Cycle threshold (Ct) values were evaluated in positive samples from the peak months of each wave, June 2020 and February 2021 (n=2360). Nucleic acid sequencing was performed in viral RNA extracted from 12 nasal swabs samples selected from patients with age lower than 60 years-old admitted to the São Rafael Hospital ICU in February 2021. Written informed consent was obtained. According to the manufacturer's instructions, the SARS-CoV-2 viral genome sequencing was performed by PGM Ion Torrent (Life Technologies, USA). cDNA was synthesized with the SuperScript VILO reverse transcriptase kit (Invitrogen, USA). The libraries were prepared using the Ion AmpliSeq SARS-CoV-2 assay panel, the Ion AmpliSeq library kit, and Ion Torrent PGM (Thermo Fisher Scientific). Genomes were submitted to Nextclade tool (https://clades.nextstrain.org) for quality control assessment and Pangolin tool (https://pangolin.cog-uk.io/) for SARS-CoV-2 lineage assignment. Maximum likelihood (ML) phylogenetic analysis was performed in IQ-TREE for all Gamma detected genomes and tree branch support was calculated with the Shimodaira-Hasegawa approximate likelihood ratio test (SH-aLRT), as previously described[11]. Background high quality (>29,000bp and <1% N) Brazilian Gamma sequences were obtained in the EpiCoV database in GISAID as available on March 25, 2021 (Supplementary Table S1) and aligned with

the genomes generated here in MAFFT[12]. The ML tree was visualized using the FigTree v1.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Statistical analysis

Categorical variables were compared using the Fisher test. Continuous data were presented as median and 95% CI; Mann-Whitney and Kruskal-Wallistest tests were used for comparisons between nonparametric data (Ct values and median age). P values<0.05 were considered significant. Data were analyzed with Prism software (v.9.1, GraphPad).

Results

Accompanying the evolution of the pandemic in the city of Salvador, data analysis demonstrated two waves of admission due to COVID-19 at the hospital, the first comprising May to July 2020 and the second one comprising December 2020 to February 2021. It is possible to observe a similar pattern of cumulative confirmed COVID-19 cases reported by Bahia state, Salvador and in São Rafael Hospital in Salvador, northeast Brazil (Figure 1A). There was a rapid increase of patients requiring hospitalization and intensive care treatments in February 2021 (Figure 1B).

Since May 2020 to February 2021, 2087 patients were admitted at the ICU at São Rafael Hospital, being 672 during the first wave (May-July 2020) and 943 in the second wave (December 2020 - February 2021). The median age of the patients hospitalized and admitted in ICU decreased in February (58 years) compared to May and June (66 years), reaching statistical significance (Figure 2A). A progressive increase in the percentage of patients with age lower

than 60 years-old being admitted to the ICU was observed in the second wave, differing from the demographic pattern observed in the first wave (Figure 2B).

Along with the increase in the percentage of young and middle-aged patients, there was also an increased percentage of patients entering the ICU without known comorbidities (cardiovascular diseases, hypertension, diabetes, obesity, hepatic diseases, asthma, kidney diseases and immunosuppression). Patients without comorbidities represented 32.20% of hospitalized COVID-19 patients admitted to the ICU due to COVID-19 in February 2021, compared to 15.32% in June 2020 ($p<0.0001$). Table 1 shows the frequency of patients with different comorbidities stratified by age group.

In addition to the changes in clinical and demographic profile found, possible alterations in the pattern of the results obtained in the RT-qPCR analysis were also evaluated. A significant reduction in the median Ct values from nasopharyngeal swab samples analyzed by RT-qPCR was observed comparing June 2020 to February 2021 ($p<0.0001$) (Figure 3). In the same periods, similar average values for the time between symptom onset and sample collection were observed, 4.4 days (95% CI 4.3 to 4.9 days) and 5.1 days (95% CI 4.8 to 5.4 days) for June 2020 and February 2021, respectively.

To investigate the viral diversity associated with the second COVID-19 wave in Salvador, we sequenced 12 swab samples from patients admitted to the ICU in February 2021 with age within the range of 18-59 years-old. Patients' characteristics are shown in Table 2. Five patients had no comorbidities. Half of the patients required invasive mechanical ventilation (IMV), while the other half were treated with non-invasive ventilation (NIV). Sequencing analysis identified the SARS-CoV-2 Gamma variant in all the evaluated samples. Although all sequences were classified by the Pangolin tool as Gamma with high probability, one of the

genomes presented low coverage and was not submitted to phylogenetic analysis. All the herein generated genomes are available at the EpiCov database, maintained by the GISAID initiative, with the accession codes EPI_ISL_160861 to EPI_ISL_1608171. ML phylogenetic tree revealed that nine of the 11 genomes clustered together with high support (SH-aLRT = 87), meaning that the Gamma lineage has already established local community transmission in Salvador (Figure 4). Two other highly supported clusters of Gamma genomes isolated in Bahia state were also observed in the tree, being composed by travelers returning from Amazonas[10]. These three clusters provide evidence of multiple introductions of the Gamma lineage into Bahia state.

Discussion

Here we report the identification of P.1 or Gamma variant in all sequenced clinical samples from patients admitted in the ICU in February 2021, in Salvador, Brazil. These results suggest that Gamma is responsible for a large share of the COVID-19 cases in Salvador, in line with different reports finding a high proportion of Gamma across Brazil[13].

Recently, researchers reported a significant increase in case fatality rates among young and middle-age adults in Parana, Brazil which may be associated with the Gamma strain[16]. The data presented herein also gives support to a role of variant Gamma in the acceleration of the pandemic in Salvador and Bahia state seen in early 2021.

It is notable the observed increase in the percentage of patients being admitted in the ICU with no risk factors for severe COVID-19, such as increased age, hypertension, diabetes, among others[16]. This data, however, is preliminary and more studies are necessary and urgent to clarify if changes in the virulence may be attributable to Gamma.

Also, it is necessary to investigate the influence of vaccine roll out, which started in mid-January, first recruiting healthcare workers and progressing to elderly individuals with age superior to 80 years-old by the end of February, utilizing either Coronavac, a two dose, 28-day interval vaccine scheme or Astrazeneca's ChAdOx nCoV-19, a two dose, 3-month interval vaccine scheme. Therefore, during the period evaluated in this study, a very low percentage of elderly patients had received two doses of the vaccine.

Previous studies have suggested increased Gamma viral load in nasopharyngeal swab samples compared to previous SARS-CoV-2 lineages[3]. Our study demonstrated that positive swab samples in February 2021 presented lower Ct values than samples evaluated in June 2020. Although Ct values are subjected to intrinsic sample collection variability and do not utilize reference measurements, the comparison of the results found in the peaks of the first wave and the second wave suggest that patients presented increased viral load in February 2021, which was also reported when Gamma samples were compared to non- Gamma samples[17,7].

Confirming that Gamma is a highly transmissible variant, associated with increased viral replication and disease severity would require restrictive control measures to be revisited in populations with confirmed Gamma circulation, to adequately prevent viral spread and pressure to the healthcare system, as has been observed in Brazil. The data presented herein does not give support to an increase mortality rate among hospitalized COVID-19 patients after the emergency of Gamma, since mortality rates and IMV rates decreased in the evaluated period. Since this data may be influenced by other confounders, such as improved disease management and evolving therapeutic protocols, future studies properly designed to answer whether Gamma may be associated with any changes in disease severity.

In summary, our findings contribute to describe the characteristics of the patients with severe COVID-19 during the second wave, which appears to be associated with an increased proportion of young and middle-aged adults. Additionally, suggests that the Gamma variant may have rapidly spread in Salvador, Brazil, leading to increased number of cases, hospitalization and ICU admissions shortly after its first detection in Bahia state. These preliminary findings reinforce the immediate need to adopt measures to reduce its spread and more rapid vaccination.

Conflict of interest statement

The authors claim no conflict of interest.

Funding

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Ethical Statement

This study was reviewed by local IRBs and received ethical approval by the National Committee for Ethics on Research (CONEP; CAAE: 29496920.8.0000.5262, 46821621.5.0000.0048 and 3.980.128 /2020). All sampled patients have provided written informed consent.

Author Contribution

Writing – Original Draft: C.K.V.N., B.S.F.S, T.G., C.A.G.R.; Writing – Review & Editing: C.K.V.N., B.S.F.S, T.G., C.A.G.R., A.V.A.M., I.C.S.; Conceptualization: B.S.F.S. and A.V.A.M.; Investigation: C.K.V.N., T.G., C.A.L.G., V.F.C., J.L.O., R.H.P., C.A.G.R, I.N.B.,

M.C.B.S., I.M.S., K.A.F.S.; Methodology: C.K.V.N., T.G., C.A.L.G., V.F.C., J.L.O., R.H.P., C.A.G.R, I.N.B., M.C.B.S., I.M.S., K.A.F.S., T.G.L.W.; Formal Analysis: C.K.V.N., T.G. and B.S.F.S.; Funding Acquisition: B.S.F.S.).

Access to data

C.K.V.N and A.V.A.M have full access to the data and are the guarantor for the data. Data is available upon reasonable request.

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Figure Legends

Figure 1. Confirmed COVID-19 hospitalizations. (A) Cumulative confirmed COVID-19 cases in Bahia state, Salvador and admitted to the São Rafael Hospital (HSR) since May 2020. (B) The total number of patients hospitalized (n=4164, blue line) and admitted to intensive care units (n=2087, orange line) from May 2020 to February 2021.

Figure 2. Temporal changes in the demographic profile of the patients admitted to the ICU due to COVID-19. (A) Individual age values (≥18-year-old) are represented along with median and 95%CI. The 1st wave includes the months of May, June, and July 2020. The 2nd wave includes the months of December 2020, January, and February 2021. (B) Total number of patients admitted to the ICU stratified by age. $^{**}p<0.001$ $^{***}p<0.0001$.

Figure 3. Temporal change in the values of SARS-CoV-2 RT-PCR cycle threshold (Ct). N gene Ct values were evaluated and compared between samples of COVID-19 referred to the hospital in June 2020 (n=1589) and February 2021 (n=771). Single Ct values and median are plotted. ****p<0.0001.

Figure 4. Circular ML phylogenetic tree of the P.1 (Gamma) lineage diversity in Brazil. Samples isolated in Bahia are shown with circles and colors represent the origin of the sample, when available. Data about traveling history is according to Tosta et al., 2021[10]. Three clusters with samples isolated in Bahia are highlighted and SH-aLRT support is shown. The tree was rooted in the oldest P.1 sampled genome.

Table 1. Comorbidity assessment in patients entering the ICU during the first wave (Jun/2020) and second wave (Feb/2021) stratified by age group

	18-29 years			30-39 years			40-49 years			50-59 years			≥ 60 years		
	Admission period			Admission period			Admission period			Admission period			Admission period		
Comorbidities	June/ 2020 (n=3)	Feb/ 2021 (n=10)	p-value*	June/ 2020 (n=19)	Feb/ 2021 (n=40)	p-value*	June/ 2020 (n=17)	Feb/ 2021 (n=66)	p-value*	June/ 2020 (n=29)	Feb/ 2021 (n=41)	p-value*	June/ 2020 (n=118)	Feb/ 2021 (n=123)	p-value*
Obesity	-	20.0%	0.999	31.6%	22.5%	0.528	41.2%	10.6%	0.007†	17.2%	19.5%	0.999	16.9%	9.8%	0.1
Cardiovascular	-	-		26.3%	12.5%	0.266	47.0%	24.2%	0.078	48.3%	51.2%	0.999	85.6%	69.1%	0.002†
Hematological	-	-		-	2.5%	0.999	-	-		3.4%	-	0.414	3.4%	1.6%	0.439
Liver disease	-	-		5.3%	-	0.322	-	4.5%	0.999	-	-		2.5%	-	0.116
Asthma	-	-		5.3%	-	0.322	11.8%	1.5%	0.105	-	-		3.4%	3.3%	0.999
Diabetes	-	-		-	2.5%	0.999	17.6%	15.2%	0.724	27.6%	20.0%	0.461	44.9%	40.5%	0.49
Neurological	-	10.0%	0.999	10.5%	-	0.999	-	-		-	2.4%	0.999	13.5%	9.8%	0.357
Lung disease	-	-		-	-		-	-		-	-		6.8%	4.1%	0.351
Immunosuppression	-	-		-	-		5.9%	-	0.205	6.9%	2.4%	0.566	3.4%	1.6%	0.439
Kidney disease	-	-		-	-		17.6%	3.0%	0.056	13.8%	4.9%	0.224	17.8%	5.7%	0.003†

*p-value of the Chi-square test or Fisher's exact test. †: P-values < 0.05.

Table 2. Clinical and demographic characteristics of patients admitted to the ICU in Feb/2021 selected for sequencing

Sex	Age (years)	Symptom onset (days before ICU admission)	Ventilatory Support	Comorbidities	Thorax CT - extension of ground-glass opacities
Male	35	7	NIV	None	25%
Male	31	7	NIV	Obesity, hypertension, diabetes	25-50%
Male	41	9	NIV	None	25-50%
Male	59	11	NIV	Obesity, hypertension	40%
Male	37	10	IMV	Obesity	25-50%
Male	46	9	IMV	Obesity	>75%
Male	44	5	IMV	None	50%
Male	41	10	NIV	None	75%
Male	56	8	IMV	Hypertension	>75%
Female	36	5	NIV	Obesity	50-75%
Male	24	8	IMV	Obesity	25-50%
Male	35	7	IMV	None	50%

CT: computed tomography; ICU: intensive care unit; NIV: non-invasive ventilation; IMV: invasive mechanical ventilation.

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