Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
SARS-CoV-2 reinfection in a healthcare professional in inner Sao Paulo during the first wave of COVID-19 in Brazil

Carlos Henrique Camargoa,*, Claudia Regina Gonçalvesa, Erica Valessa Ramos Gomes Pagnocaa, Janaina Olher Martins Montanhab, Maricélia Navarro Pinheiro Floresb, Márcia Maria Costa Nunes Soaresb, Fernanda Modesto Tolentino Binhardib, Patrícia Marques Ferreirac, Ana Lucia Frugis Yuc, Telma Regina Marques Pinto Carvalhanasc, Adriano Abbudb, Adriana Bugnoa, Claudio Tavares Sacchia

Instituto Adolfo Lutz, Laboratório Central, São Paulo, Brazil
b Instituto Adolfo Lutz, Centro de Laboratório Regional, São José do Rio Preto, São Paulo, Brazil
c Divisão de Doenças de Transmissibilidade Respiratórias, Centro de Vigilância Epidemiológica, Secretaria de Estado da Saúde, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 20 February 2021
Revised in revised form 14 July 2021
Accepted 28 July 2021
Available online 20 August 2021

ABSTRACT

Coronavirus Disease 2019 pandemic remains a threat to public health. We report 2 cases of Coronavirus Disease 2019 infection in the same healthcare professional in Brazil. Genomic analysis identified that primary infection was caused by the endemic lineage B.1.1.33 while reinfection by the lineage B.1.1.44, a lineage with an additional V1176F mutation in S protein.

© 2021 Elsevier Inc. All rights reserved.

Keywords:
Coronavirus Disease 2019
SARS-CoV-2
Reinfection
COVID-19

1. Case report

Coronavirus Disease 2019 (COVID-19), caused by severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) emerged in China and rapidly evolved to a Public Health Emergency of International Concern (Harapan et al., 2020). Until end of 2020, >75 millions of cases and >1.5 millions of deaths were reported worldwide (Dong et al., 2020).

Phylogenetic studies report different lineages of SARS-CoV-2 simultaneously circulating in a given region with particular specificities (Mercatelli and Giorgi, 2020), mainly associated with neutral mutations not associated with enhanced transmissibility (van Dorp et al., 2020). According to Global Initiative on Sharing All Influenza Data (GISAID) database, in Brazil, the most common concurrently circulating lineages are B.1.1.28, B.1.1.33 and P.1 and P.2 lineages, variants of concern derivatives of B.1.1.28.

Although cases of reinfection are still rare (Larson et al., 2020, Mulder et al., 2020, Prado-Vivar et al., 2020, Tillett et al., 2020), weak or absent immunological response to SARS-CoV-2 after natural infection can occur (Wu et al., 2020), which may contribute to reinfections as also observed with other seasonal coronavirus (Edridge et al., 2020).

The epidemiologic definition of reinfection considers the presence of distinct phylogenetic lineages in an interval of >90 days, and low CT in RT-PCR assays (Yahav et al., 2020). Here, we report a confirmed episode of SARS-CoV-2 reinfection in a patient from Brazil.

All biological samples suspected to be from cases of reinfection are sent to Central Instituto Adolfo Lutz Laboratory for further analysis and confirmation. For those samples, the RNA was reextracted using the automatic Extracta 32 (Loccus, Brazil) and the presence of E, N, RdRP and RNAseP genes were confirmed using the Allplex 2019-nCoV Assay (Korea, Seegene Inc.) on the QuantStudio 5 Real-Time PCR Systems (Applied Biosystems, USA).

For genome sequencing, AmpliSeq™ SARS-CoV-2 (Thermo Fisher Scientific Inc., USA) was employed. Next, analysis were performed after quality control checking, assembly by IRMA (https://wonder.cdc.gov/amd/flu/irma/) and phylogenetics analysis in the GISAID database and BioNumerics 8.0 software (Applied Maths, Sint-Martens-Latem, Belgium). Sequences were deposited in the GISAID database under the accession IDs EPI_ISL_708529 and EPI_ISL_708530 and compared with reference WIV04-sequence (EPI_ISL_402124).
and shortness of breath were reported 30 days after the diagnosis of SARS-CoV-2. Chest x-ray did not show abnormalities. Persistent fatigue, headache, and diarrhea were also reported. Temperature of 36.6°C and O2 saturation of 98% were measured. On December 10, 2020, the Central Laboratory received 2 biological samples from a female nursing assistant, aged 41 years old, living in Fernandopolis, Sao Paulo, Southeast Brazil. Patient did not report comorbidities except for a previous gastroplasty.

On November 21, 2020, after the patient presented again with symptoms of headache, cough, tiredness and myalgia, a second sample was collected and positive for SARS-CoV-2. Complete aligned sequences of SARS-CoV-2 of 29,852bp and 29,862bp genomes with a coverage of 15,677x and 15,848x were obtained. GISAID classified these 2 genomes into 2 different lineages (as classified on January 22, 2021): B.1.1.33 in the primoinfection and B.1.1.44 in reinfection. Both lineages belong to Nexitrain clade 20B, but in different subbranches (Supplementary Figure).

In comparison with the WIV04-sequence, mutations were identified in S, N and NSP proteins of both genomes, but reinfection case (lineage B.1.44) presented an additional V1176F mutation in S protein (Table 1). In addition, both sequences presented the R203K and G1219C aminoacid change in the nucleocapsid (N) protein. On the other hand, only the EPI_ISL_708529 sequence presented the I292T aminoacid change in the envelope (E) protein. In comparison with the WIV04-sequence (EPI_ISL_402124), we found that the average value of mutations in S protein of 19, 19, 22, and 26 for E, N, RdRP and RNAseP genes on the first sample and 17, 17, 19, and 25 for the same genes on the second sample, respectively.

Also, GISAID database was analyzed to compare the mutations present in S protein in all reinfection cases available (as of January 22, 2021). Complete aligned sequences of SARS-CoV-2 of 29,852bp and 29,862bp genomes with a coverage of 15,677x and 15,848x were obtained. GISAID classified these 2 genomes into 2 different lineages (as classified on January 22, 2021): B.1.1.33 in the primoinfection and B.1.1.44 in reinfection. Both lineages belong to Nexitrain clade 20B, but in different subbranches (Supplementary Figure).

In comparison with the WIV04-sequence, mutations were identified in S, N and NSP proteins of both genomes, but reinfection case (lineage B.1.44) presented an additional V1176F mutation in S protein (Table 1). In addition, both sequences presented the R203K and G204R aminoacid change in the nucleocapsid (N) protein. On the other hand, only the EPI_ISL_708529 sequence presented the I292T and P383L in N protein, besides an I33T change in NS6 - Accessory protein 6 (NS6). Mutations found only in EPI_ISL_708530 included L54F, D614G, H655Y, T1027I, V1176F. Analyzing the publicly available sequences of infection and reinfection episodes, we found that the average value of mutations in S protein in

Table 1
Clinical and genomic characteristics of SARS-Cov-2 infection and reinfection cases in Brazil.

| Parameter                  | First episode – primo infection | Second episode – reinfection |
|----------------------------|---------------------------------|-----------------------------|
| Collection date            | June 29, 2020                   | November 21, 2020           |
| Symptoms                   | headache, myalgia, nonproductive cough, shortness of breath, ageusia and anosmia | headache, myalgia, nonproductive cough, fever, diarrhea, appetite loss, dizziness, and chest pain |
| SARS-CoV-2 lineage         | B.1.1.33                        | B.1.1.44                    |
| GISAID Accession ID        | EPI_ISL_708529                  | EPI_ISL_708530              |
| Average coverage           | 15.77x                          | 15.848x                     |
| Genetic differences         |                                 |                             |
|                           |                                 |                             |

In comparison with WIV04-sequence (EPI_ISL_402124).

| Case | Patient gender/age | Country | Type               | Collection date | Accession ID | Spike protein mutations |
|------|--------------------|---------|--------------------|-----------------|--------------|------------------------|
| 1    | Female/41          | Brazil  | primo infection     | 2020/06/29      | EPI_ISL_708529 | D614G                 |
| 2    | Female/29          | Brazil  | primo infection     | 2020/10/26      | EPI_ISL_708529 | D614G, V1176F          |
| 3    | Female/37          | Brazil  | primo infection     | 2020/10/13      | EPI_ISL_708529 | D614G                 |
| 4    | Male/unknown       | Ecuador | primo infection     | 2020/06/01      | EPI_ISL_708529 | D614G, G1219C          |
| 5    | Female/45          | Brazil  | primo infection     | 2020/10/26      | EPI_ISL_708529 | D614G, V1176F          |
| 6    | Female/57          | Brazil  | primo infection     | 2020/05/20      | EPI_ISL_708529 | D614G                 |
| 7    | Male/34            | Brazil  | primo infection     | 2020/05/29      | EPI_ISL_708529 | D614G                 |
| 8    | unknown            | USA     | primo infection     | 2020/04/18      | EPI_ISL_708529 | D614G                 |
| 9    | unknown            | Netherlands | primo infection     | 2020/04/06      | EPI_ISL_708529 | D614G                 |
| 10   | Male/33            | Hong Kong | primo infection     | 2020/06/08      | EPI_ISL_708529 | D614G                 |
| 11   | Male/51            | India   | primo infection     | 2020/10/24      | EPI_ISL_708529 | D614G                 |

In comparison with WIV04-sequence (EPI_ISL_402124).

Cases reported in this study.
reinfections episodes was numerically higher than those in the primary infections (2.82 vs 1.09, respectively; \( P = 0.0974 \)) (Table 2).

Detection of reinfection in a patient from Brazil underscores the continuous threat of COVID-19 in a country suffering from uncontrolled epidemic about to be exacerbated by a second disease wave. Although clinical significance of COVID-19 reinfection remains to be totally elucidated, apprehensions clearly arise from the possibility that, in at least some individuals, the immunological response may be not enough to prevent a second infection.

The reinfection case presented here is supported by the fact that clinical specimens were collected more than 90 days apart from the same symptomatic patient presenting positive results with low CT values for SARS-CoV-2. Moreover, the first episodes was caused by a common lineage circulating in Brazil, B.1.1.33, which is highly disseminated in the country, according to GISAID database (Rambaut et al., 2020). B.1.1.44 is reported exclusively in this case in Brazil but another 616 entries (mainly from Europe) are deposited in the GISAID repository. Lineage B.1.1.44 was previously characterized as B.1.1.248 and B.1.1.28, another recurrent lineage circulating in Brazil since April, which originated the variants of concern P.1 (Gamma) and P.2, in increasing prevalence (Sabino et al., 2021). A remarkable difference found in B.1.1.44 is an additional V176F substitution besides the highly disseminated D614G amino acid substitution prevalent worldwide (Rahimi et al., 2020); increased number of mutations in S protein are also reported in other reinfection episodes (Table 2). Mutation in the spike S2 domain V176F is recognized to be the second more frequent in SARS-CoV-2 from South America, but its frequency is <3% in available viral genomes. Besides our epidemiological and genomic data, absence of patient serologies against SARS-CoV-2 cannot be ignored as potential limitation in this study.

Finally, we report a reinfection case of COVID-19 in a patient from Brazil, demonstrated by genome sequencing, highlighting the potential of Brazilian lineages (and its derivatives) in causing reinfection. The impact of passive immunization on lineages circulation and effective protection against COVID-19 reinfection, however, deserves further evaluation.

Funding

This study was funded by Coordenação de Controle de Doenças (CCD), Secretaria de Estado da Saúde de São Paulo, Brazil and by the grants 2017/50333-7, 2018/21193-5, Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

Authors’ contributions

Conceptualization: AA, AB, CTS
Data collection: CRG, EVRGP, KRC, JOMM, MNPF, MMC, FMB, ALFY, TRMPC
Data analysis: CHC, AA, AB, CTS
Writing – original draft: CHC, CTS
Writing and Review and Editing: CHC, AA, AB, CTS

Data availability

Sequences were deposited in the GISAID database under the accession IDs EPI_ISL_708529 and EPI_ISL_708530.

Ethical approval

This study was approved by the Local Ethics Committee (CAAE 139 75713020.7.0000.0059).

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Acknowledgments

We are thankful to staff dedicated to COVID-19 diagnostic and control, namely those from Coordenação Científica de Laboratórios de Saúde Pública (CCLAB), Coordenação de Controle de Doenças (CCD) from Secretaria de Estado da Saúde de São Paulo.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.diagmicrobio.2021.115516.

References

Dong E, Du H, Gardner L. An interactive web-based dashboard to track COVID-19 in real time. Lancet Infect Dis 2020;20:533–4.
Edridge AWD, Kaczorowska J, Hoste ACR, Bakker M, Klein M, Loens K, et al. Seasonal coronavirus protective immunity is short-lasting. Nat Med 2020;26:1691–3. Epub ahead of print.
Harapan H, Itoh N, Yufika A, Winardi W, Kean S, Te H, et al. Coronavirus disease 2019 (COVID-19): a literature review. J Infec Public Health 2020;13:667–73.
Larson D, Brodin SK, Voegly LJ, Cet RZ, Glang IA, Malagon P, et al. A case of early reinfection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Clin Infect Dis 2020. doi: 10.1093/cid/ciaa1436. Epub ahead of print.
Mercatelli D, Giorgi PM. Geographic and genomic distribution of SARS-CoV-2 mutations. Front Microbiol 2020;11:1800.
Mulder M, van der Vegt DSM, Oude Munnink BR, Geurtsvan Kessel CH, van de Bovenkamp J, Sikkema RS, et al. Reinfection of severe acute respiratory syndrome coronavirus 2 in an immunocompromised patient: a case report. Clin Infect Dis 2020. doi: 10.1093/cid/ciaa1538. Epub ahead of print.
Prado-Vivar B, Becerra-Wong M, Guadalupe JJ, Marquez S, Gutierrez B, Rojas-Silva P, et al. COVID-19 Re-Infection by a phylogenetically distinct SARS-CoV-2 variant, first confirmed event in South America. SSRN Electron J 2020. doi: 10.2139/ssrn.3686174. Epub ahead of print.
Rahimi A, Miraazadeh A, Tavakolpour S. Genetics and genomics of SARS-CoV-2: a review of the literature with the special focus on genetic diversity and SARS-CoV-2 genome detection. Genomics 2020. doi: 10.1016/j.jgyge.2020.09.059. Epub ahead of print.
Rambaut A, Holmes EC, O’Toole A, Hill V, Mc Crone JT, Ruis C, et al. A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. Nat Microbiol 2020;5:1403–7.
Sabino EC, Bues LF, Carvalho MPS, Prete CA, Crispim MAE, Frajai NA, et al. Resurgence of COVID-19 in Manaus, Brazil, despite high seroprevalence. Lancet 2021. doi: 10.1016/S0140-6736(21)01835-5. Epub ahead of print.
Tillett RL, Sevinsky JR, Hartley PD, Kerwin H, Crawford N, Goralski A, et al. Genomic evidence for reinfection with SARS-CoV-2: a case study. Lancet Infect Dis 2020. doi: 10.1016/S1473-3099(20)30764-7. Epub ahead of print.
To KK-W, Hung IF-N, Ip JD, Chu AW-H, Chan W-M, Tam AR, et al. Coronavirus disease 2019 (COVID-19) Re-infection by a phylogenetically distinct severe acute respiratory syndrome coronavirus 2 strain confirmed by whole genome sequencing. Clin Infect Dis 2020. doi: 10.1093/cid/ciaa1275. Epub ahead of print.
van Dorp L, Richard D, Tan CCS, Shaw LP, Acman M, Balloux F. No evidence for increased transmissibility from recurrent mutations in SARS-CoV-2. Nat Commun 2020. doi: 10.1038/s41467-020-19818-2. Epub ahead of print.
Wu F, Wang A, Liu M, Wang Q, Chen J, Xia S, et al. Neutralizing antibody responses to SARS-CoV-2 in an immunocompromised patient: a case report. Clin Infect Dis 2020. doi: 10.1093/cid/ciaa1275. Epub ahead of print.
Yahav D, Yelin D, Eckerle I, Eberhardt CS, Wang J, Cao B, et al. Definitions for COVID-19 reinfection, relapse and PCR re-positivity. Clin Microbiol Infect 2020. doi: 10.1016/j.cmi.2020.11.028. Epub ahead of print.