Heliyon 10 (2024) e23485

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Case Report of a Young Adult with Fatal COVID-19 and Abundant SARS-CoV-2 Nucleocapsid Protein and Lipofuscin Accumulation in Tissues

Rosalia Lira [a], César Luna-Rivero [b], Francina Valezka Morales-Bolaños [c], José Luis Sandoval-Gutiérrez [d], Elsa Romelia Moreno-Verduzco [e], Angélica Maldonado-Rodriguez [f], Jesús Miguel Torres-Flores [g], Martha Yocupicio-Monroy [h], Edgar E. Sevilla-Reyes [i,j,*]

[a] *Unidad de Investigación en Biomedicina y Oncología Genómica, Hospital Gineco Pediatría 3A, OOAD DF Norte, Instituto Mexicano del Seguro Social, Ciudad de México, Mexico*
[b] *Servicio de Anatomía Patológica, Instituto Nacional de Enfermedades Respiratorias, Ciudad de México, Mexico*
[c] *Subdirección de Cirugía Cardiotorácica, Instituto Nacional de Enfermedades Respiratorias, Ciudad de Mexico, Mexico*
[d] *Subdirección de Servicios Auxiliares de Diagnóstico, Instituto Nacional de Enfermedades Respiratorias, Ciudad de Mexico, Mexico*
[e] *Subdirección de Servicios Auxiliares de Diagnóstico, Instituto Nacional de Perinatología, Ciudad de México, Mexico*
[f] *Unidad de Investigación Médica en Enfermedades Infecciosas y Parasitarias, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, Ciudad de Mexico, Mexico*
[g] *Laboratorio Nacional de Vacunología y Virus Tropicales, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Ciudad de Mexico, Mexico*
[h] *Universidad Autónoma de la Ciudad de México, Posgrado en Ciencias Genómicas, Ciudad de Mexico, Mexico*
[i] *Centro de Investigación en Enfermedades Infecciosas, Instituto Nacional de Enfermedades Respiratorias, Ciudad de Mexico, Mexico*
[j] *Laboratorio de Transcriptómica e Inmunología Molecular, Instituto Nacional de Enfermedades Respiratorias, Ciudad de Mexico, Mexico*

ARTICLE INFO

Keywords:
COVID-19
lipofuscin
Nucleocapsid protein
Muscle
Case report

ABSTRACT

This is a case report of a young adult who died of COVID-19 twelve days after admission, with coronavirus nucleocapsid protein and lipofuscin found in the heart and kidney tissues, providing further evidence of the role of SARS-CoV-2 in cellular senescence.

1. Introduction

COVID-19 pathology extends beyond the respiratory system. Autopsy evidence shows SARS-CoV-2 in multiple organs [1] including an increased risk of heart failure [2] and cellular senescence [3], detected as an accumulation of substances like lipofuscin [4], an auto-fluorescent pigment, in muscles, neurons, skin, and kidneys of elderly people mostly. In this report, we describe the critical case of a young adult with COVID-19 who was intubated and died 12 days later. The autopsy revealed SARS-CoV nucleocapsid protein (N) in

* Corresponding author. Lab. de Transcriptómica e Inmunología Molecular, Instituto Nacional de Enfermedades Respiratorias (INER) Calz. de Tlalpan 4502. Sección XVI. CDMX 14080. Mexico.
E-mail address: edgar.sevilla@iner.gob.mx (E.E. Sevilla-Reyes).

https://doi.org/10.1016/j.heliyon.2023.e23485
Received 26 June 2023; Received in revised form 3 December 2023; Accepted 5 December 2023
Available online 10 December 2023

myocytes and kidney tissues, and lipofuscin accumulation in cardiomyocytes and kidney tissue, providing further evidence of the role of SARS-CoV-2 in cellular senescence.

1.1. Patient information

In March 2020, a 27-year-old male of Mestizo ethnicity, employed as public transport driver and resident from Mexico City, with a body mass index of 35.0, a self-reported cigarette consumption of one per day and no other significant medical history, was hospitalized in a medical unit with symptoms of urinary tract infection. One week after discharge, the patient was admitted to the INER emergency room with a 24-h history of cough with mild hemoptysis.

1.2. Clinical findings

On arrival, the patient had an oxygen saturation of 60 %, severe hypoxemia, an oxygenation index (PaO_2/FiO_2) of 183, and infiltrates/opacities observed on chest tomography on admission. The patient's heart rate was 80 beats per minute and an electrocardiogram showed sinus rhythm with no evidence of cardiac ischemia, cavity enlargement, or electrical conduction disorder.

1.3. Therapeutic intervention

The patient underwent orotracheal intubation under sedation with midazolam/fentanyl and vecuronium. Subsequently, the patient was placed in the prone position and underwent hemodialysis. Twelve days after admission and despite treatment, the patient developed septic shock with renal failure and deteriorated into irreversible cardiorespiratory arrest after CPR maneuvers. All care given was standard and there were no limitations on equipment or resources at that time.

1.4. Diagnostic assessment

COVID-19 was diagnosed using RT-PCR on the same day of arrival with a bad prognosis given by elevated creatine kinase at 568 IU/L (normal 49–397 IU/L). On admission, the patient was diagnosed with ARDS (SOFA score 17 points, APACHE II 16 points, SAPS II 55 points), lymphopenia, thrombocytopenia, and electrolytic imbalance. Since admission, the total leukocyte counts steadily increased from 7.9 (admission) to 26×10^9/L (two days before death). Further test results are available in Supplementary Table.

Biopsies were obtained using minimally invasive techniques by 8 hours after death. Specimens were processed for paraffin block

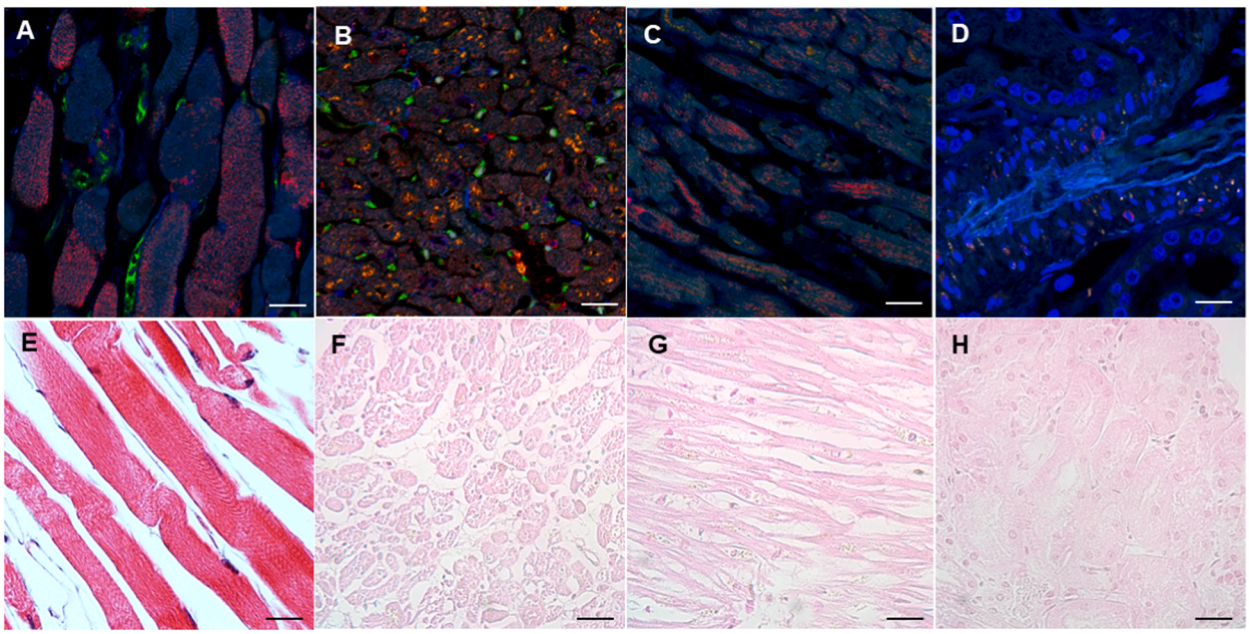

Fig. 1. SARS-CoV-2 Nucleocapsid and lipofuscin detection in tissues. N antigen was present in a punctuated pattern in skeletal fibers (panel A in red), cardiomyocytes (sagittal plane, panel B; longitudinal plane, panel C) and kidney tissue around the blood vessels (panel D). COVID-19 case skeletal muscle fibers contain larger interstitial space (panel E) where infiltrates of $CD31^+$ cells can be observed (panel A in green). COVID-19 case cardiac fibers also show increased interstitial space (panels B, C, F, G) and the N protein was irregularly distributed in the sarcoplasma (panels B and C in red). Tissues stained for IIF (panels A–D) for SARS-CoV nucleocapsid protein (red), $CD31^+$ (green), nuclei (blue), and H&E (panels E–H). Bar size = 20 μm.

fixation. Sections of 5 μm were stained either with hematoxylin and eosin (H&E) or for indirect immunofluorescence (IIF). Deparaffinized slides were incubated overnight with 1:400 anti-SARS-CoVs nucleocapsid antibody (NB100-56576, Novus Biologicals) and 1:100 anti-CD31/PECAM-1 antibody (NB600-562, Novus Biologicals). Incubated with a 1:500 goat anti-rabbit IgG H&L-Alexa Fluor 555 (Abcam, ab150078) and Goat Anti-Mouse IgG H&L-Alexa Fluor 488 (Abcam, ab150113), and analyzed by confocal microscopy (Nikon).

The SARS-CoV-2 N protein was abundantly detected in the lung (Supplementary Fig 1 (**previously 2) multiple skeletal muscle fibers, distributed throughout the sarcoplasm in a distinctive punctuated pattern, and a minor accumulation towards the sarcolemma (Fig. 1A). Structurally, skeletal muscle cells exhibited an increased interstitial space (Fig. 1E) compared to uninfected tissues (Supplementary Fig. 1), with many $CD31^{+}$ infiltrating cells (Fig. 1A).

The N protein was also detected in the sarcoplasm of the cardiac tissue, but the distribution was distinctive: it was concentrated in heterogeneous clusters (Fig. 1B and C). The cardiac muscle tissue showed minimal structural changes, apart from the increased interstitial space between the fibers (Fig. 1F and G). Another characteristic feature was the extensive lipofuscin accumulation (Fig. 1B, C, 1G and Supplementary Fig. 3) in some regions of the cardiac tissue of the young adult. Similarly, the kidney tissues were positive for SARS-CoV-2 N protein and lipofuscin (Fig. 1D), mainly around the tunica externa of the blood vessel (Supplementary Fig. 4). Other tissues such as the aorta, liver, and pulmonary vein were negative for N and lipofuscin (Supplementary Fig. 5), although limited cross reactivity was observed particularly in the pulmonary vein (Supplementary Fig. 5A).

2. Discussion

Here, we report an autopsy case of a young obese adult deceased with COVID-19, early during the pandemic and before vaccination was available, with the accumulation of SARS-CoV-2 N-antigen in the sarcoplasm of skeletal and cardiac muscle fibers as well as in the kidney tissue, together with lipofuscin accumulation in myocytes and kidney tissues. The patient had a poor prognosis upon arrival, due to obesity, a light smoking history, the requirement of invasive mechanical ventilation and elevated creatine kinase values. Because of these dates, the patient most probably acquired COVID-19 in another medical unit and developed pneumonia well before arriving at INER, making this a likely case of healthcare-associated COVID-19 infection, with the worst clinical outcome.

SARS-CoV-2 antigens are found extrapulmonary in postmortem cases or biopsies [5,6] even after the virus is cleared from the nasopharynx [7]. In a cohort of patients aged approximately 60 years, replicating viral RNA was present in cardiomyocytes without apoptosis, and the SARS-CoV-2 N antigen co-localized with sarcoplasmic alpha-actin [8]. N-protein in cardiac myofibrils has been reported [8,9] but its detection in skeletal tissues is rare, despite the significant muscular pain and myositis in COVID-19. Creatine kinase, a known biomarker for muscular damage and a predictor of COVID-19 severity [10], was elevated on admission in the case described here, indicating a higher likelihood of severe disease and muscular damage, which was not identified in the examined sample, but we cannot discard that other muscles could have been affected.

Lipofuscin is a marker for oxidative stress and cellular senescence [4]. COVID-19 causes thrombosis and inflammation, which are associated with severe oxidative stress, conditions where lipofuscin is known to increase. Pathogen-induced lipofuscinosis and virus-induced cellular senescence [11,12] are phenomena that have been described in COVID-19 cases in older adults. In this case, lipofuscin was abundant in the myocardial and kidney tissues of a young person, specifically in tissues where the coronavirus N antigen was also present. In the kidney, lipofuscin appeared localized in the outer layer of the blood vessel (tunica externa). As lipofuscin was absent from the examined liver tissues, where it is commonly present, we can speculate that this phenomenon is possibly linked to SARS-CoV-2 N expression rather than a systemic feature of the patient.

Another feature of this case is that the N clusters in cardiomyocytes were distinct, irregular, and wider than those reported previously [8]. Further analyses of other cases are required to verify this presentation and clarify whether the autopsy timing might have affected this observation.

This case report has certain limitations to consider: the extended timing of the autopsy and the unavailability of a more complete medical history of the patient, which might influence the data and the interpretations presented here. Further, given the nature of a minimally invasive autopsy, we cannot exclude that other regions in the liver and other regions may show lipofuscin accumulation.

Although the patient was treated with multiple myotoxic and nephrotoxic drugs, our data suggest that SARS-CoV-2 N protein and lipofuscin accumulation are part of the viral pathology, as they are expressed together in the kidneys and cardiomyocytes only.

We provide evidence regarding the SARS-CoV-2 effects on muscular and kidney tissues suggesting that the virus may contribute to damage and cellular senescence. Particularly, the risk of cardiovascular disease increases after COVID-19, that is proportional to the disease severity. Future prospective studies should include the determination of viral nucleocapsid antigen and antibodies in serum that could help to clarify their role in the pathogenesis of the disease.

This autopsy case of a young obese adult deceased with COVID-19, early during the pandemic and before vaccination was available, shows the extensive accumulation of SARS-CoV-2 N-antigen in the sarcoplasm of skeletal and cardiac muscle fibers and kidney tissue, together with lipofuscin accumulation in myocytes and kidney tissues, providing evidence of its potential to cause cellular senescence in the tissues of a young adult.

3. Ethics statement

This protocol was approved by the Biosafety, Bioethics, and Research Institutional Committee of the INER (protocol number E02-21). Before the minimally invasive autopsy, informed consent was obtained from the next of kin for the publication of all images, clinical data and other data included in the main manuscript.

CARE guidelines.

To the best of our abilities, the CARE checklist has been covered, however there are some obvious aspects that were left out such as 5d) Relevant past outcomes and interventions (due to the novel pathogen, there was no previous intervention on this patient), 9b) administration of therapeutic intervention (given the focus of the case report was considered irrelevant as no specific treatment was available at that time and drugs were generic sedatives, analgesic and neuromuscular blockers), 10c) adherence and tolerability (given that there was no specific treatment), 10d) Adverse or unanticipated events (given the prognostic at admission, this was not elaborated any further), 12) patient perspective (deceased).

Funding

This work was supported by Instituto Nacional de Enfermedades Respiratorias and Instituto Nacional de Perinatología in Mexico.

Data availability statement

All relevant data for this case report has been included in the main article and in the supplementary material files.

CRediT authorship contribution statement

Rosalia Lira: Writing – review & editing, Investigation, Formal analysis, Conceptualization. **César Luna-Rivero:** Writing – review & editing, Investigation, Data curation. **Francina Valezka Morales-Bolaños:** Writing – review & editing, Investigation, Data curation. **José Luis Sandoval-Gutiérrez:** Writing – review & editing, Validation, Supervision, Methodology, Data curation. **Elsa Romelia Moreno-Verduzco:** Writing – original draft, Validation, Investigation, Conceptualization. **Angélica Maldonado-Rodriguez:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Jesús Miguel Torres-Flores:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis. **Martha Yocupicio-Monroy:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis. **Edgar E. Sevilla-Reyes:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Vadim Pérez Koldenkova for his support with the confocal microscopy at the Laboratorio Nacional de Microscopía Avanzada/Centro Médico Nacional Siglo XXI, IMSS, and María Esther Jaime Capetillo for her support in retrieving clinical laboratory data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23485.

References

[1] A. Deroubaix, A. Kramvis, Imaging techniques: essential tools for the study of SARS-CoV-2 infection, Front. Cell. Infect. Microbiol. 12 (2022), 794264, https://doi.org/10.3389/fcimb.2022.794264.
[2] M. Zuin, G. Rigatelli, L. Roncon, G. Pasquetto, C. Bilato, Risk of incident heart failure after COVID-19 recovery: a systematic review and meta-analysis, Heart Fail. Rev. (2022) 1–6, https://doi.org/10.1007/s10741-022-10292-0.
[3] C.A. Schmitt, T. Tchkonia, L.J. Niedernhofer, P.D. Robbins, J.L. Kirkland, S. Lee, COVID-19 and cellular senescence, Nat. Rev. Immunol. 23 (2023) 251–263, https://doi.org/10.1038/s41577-022-00785-2.
[4] K. Evangelou, D. Veroutis, K. Paschalaki, P.G. Foukas, N. Lagopati, M. Dimitriou, et al., Pulmonary infection by SARS-CoV-2 induces senescence accompanied by an inflammatory phenotype in severe COVID-19: possible implications for viral mutagenesis, Eur. Respir. J. 60 (2022), https://doi.org/10.1183/13993003.02951-2021.
[5] J. Liu, Y. Li, Q. Liu, Q. Yao, X. Wang, H. Zhang, et al., SARS-CoV-2 cell tropism and multiorgan infection, Cell Discov 7 (2021) 1–4, https://doi.org/10.1038/s41421-021-00249-2.
[6] A. Roshdy, S. Zaher, H. Fayed, J.G. Coghlan, COVID-19 and the heart: a systematic review of cardiac autopsies, Frontiers in Cardiovascular Medicine 7 (2021).
[7] C.C.L. Cheung, D. Goh, X. Lim, T.Z. Tien, J.C.T. Lim, J.N. Lee, et al., Residual SARS-CoV-2 viral antigens detected in GI and hepatic tissues from five recovered patients with COVID-19, Gut 71 (2022) 226–229, https://doi.org/10.1136/gutjnl-2021-324280.
[8] G.P. Bulfamante, G.L. Perrucci, M. Falleni, E. Sommariva, D. Tosi, C. Martinelli, et al., Evidence of SARS-CoV-2 transcriptional activity in cardiomyocytes of COVID-19 patients without clinical signs of cardiac involvement, Biomedicines 8 (2020) 626, https://doi.org/10.3390/biomedicines8120626.
[9] J. Suh, S.S. Mukerji, S.I. Collens, R.F. Padera, G.S. Pinkus, A.A. Amato, et al., Skeletal muscle and peripheral nerve histopathology in COVID-19, Neurology 97 (2021) e849–e858, https://doi.org/10.1212/WNL.0000000000012344.

[10] S.A. Friedman, Z. Charmchi, M. Silver, N. Jacoby, J. Perk, Y. Anziska, Skeletal muscle manifestations and creatine kinase in COVID-19, Neurohospitalist 12 (2022) 597–606, https://doi.org/10.1177/19418744221105961.
[11] L.L. Williams, H.R. Sloan, Lipofuscin in rabbit skin: its occurrence after ocular herpes simplex infection, Arch. Pathol. Lab Med. 107 (1983) 40–45.
[12] A.V. Pereira, M.B. Gois, M.S. Silva, NR de Miranda Junior, C.B.H.F. Campos, L.C.L. Schneider, et al., Toxoplasma gondii causes lipofuscinosis, collagenopathy and spleen and white pulp atrophy during the acute phase of infection, Pathog Dis 77 (2019), https://doi.org/10.1093/femspd/ftaa008 ftaa008.