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COVID-19 Endothelial Dysfunction Can Cause Erectile Dysfunction: Histopathological, Immunohistochemical, and Ultrastructural Study of the Human Penis

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Purpose: A pilot study to describe histopathological features of penile tissue of patients who recovered from symptomatic COVID-19 infection and subsequently developed severe erectile dysfunction (ED).

Materials and Methods: Penile tissue was collected from patients undergoing surgery for penile prosthesis for severe ED. Specimens were obtained from two men with a history of COVID-19 infection and two men with no history of infection. Specimens were imaged with TEM and H&E staining. RT-PCR was performed from corpus cavernosum biopsies. The tissues collected were analyzed for endothelial Nitric Oxide Synthase (eNOS, a marker of endothelial function) and COVID-19 spike-protein expression. Endothelial progenitor cell (EPC) function was assessed from blood samples collected from COVID-19 (+) and COVID-19 (-) men.

Results: TEM showed extracellular viral particles ~100 nm in diameter with peplomers (spikes) near penile vascular endothelial cells of the COVID-19 (+) patients and absence of viral particles in controls. PCR showed presence of viral RNA in COVID-19 (+) specimens. eNOS expression in the corpus cavernosum of COVID-19 (+) men was decreased compared to COVID-19 (-) men. Mean EPC levels from the COVID-19 (+) patients were substantially lower compared to mean EPCs from men with severe ED and no history of COVID-19.

Conclusions: Our study is the first to demonstrate the presence of the COVID-19 virus in the penis long after the initial infection in humans. Our results also suggest that widespread endothelial cell dysfunction from COVID-19 infection can contribute to ED. Future studies will evaluate novel molecular mechanisms of how COVID-19 infection leads to ED.

Keywords: COVID-19; Endothelium; Erectile dysfunction; Histopathology; Immunohistochemistry; SARS-CoV-2

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) was originally observed in China, in December 2019, and since then has grown into a global pandemic [1]. Studies have shown that the ability of COVID-19 to enter cells relies heavily on the presence of angiotensin converting enzyme-2 (ACE-2). Before binding to ACE-2 receptors, viral spike proteins must be primed by cellular proteases, specifically, transmembrane protease serine 2 (TMPRSS-2) [2]. Therefore, COVID-19 appears to affect cells and tissues that co-express ACE-2 and TMPRSS-2 [3]. Interestingly, both the ACE-2 receptor and *TMPRSS-2* gene are expressed on endothelial cells and likely explains why COVID-19 infection produces widespread endothelial dysfunction. Electron microscopy has demonstrated the presence of COVID-19 viral elements in endothelial cells of affected organs, such as the lung, heart, and kidney. These findings raise the question as to whether erectile tissue in the penis, rich in endothelial lined blood vessels, can also be subject to wide-spread endothelial dysfunction caused by COVID-19. Here we describe the histopathological features of penile tissue of patients who recovered from symptomatic COVID-19 infection and subsequently developed severe erectile dysfunction (ED).

MATERIALS AND METHODS

After providing informed consent, penile tissue was collected from patients undergoing surgery for penile prosthesis surgery due to severe ED under an Institutional Review Board of the University of Miami Miller School of Medicine approved protocol (IRB No: 20150740). Patients' ages ranged from 65 to 71 years old, and all were of Hispanic ethnicity. Two specimens were obtained from males with a history of COVID-19 infection and two specimens were obtained from men with no history of infection (all men tested negative for COVID-19 *via* PCR at least 24 hours before the operation). Tissue from COVID-19 (+) and COVID-19 (-) specimens were imaged using transmission electron microscopy (TEM) and stained with hematoxylin and eosin (H&E).

The tissue was analyzed for viral RNA using polymerase chain reaction (PCR). Total RNA was isolated from penis tissue biopsies using TRIzol (Ambion catalog #15596018; Invitrogen, Carlsbad, CA, USA). Quantitation of mRNAs was performed using BIORAD Gene Expression Assays according to the manufacturer's protocol. RNA was reverse transcribed to complementary DNA using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol by thermal cycler instrument (BioRad, Hercules, CA, USA). Quantitative real time PCR (RT-PCR) was performed to detect the presence of COVID-19 genes using the BioRad® Real-Time PCR system (BioRad). The total volume of the RT-PCR reaction was 20 μL, containing 10 μL SYBR Universal PCR Master Mix (BioRad), 2 μL Primer mix (forward primer and reverse primer), 2 μL PCR product as the template, and 6 μL dH_2O. Each sample was tested in technical triplicate and the specificity of the reaction was determined by melting curve analysis at the dissociation stage. The sequences of 2019-Novel Coronavirus RT-PCR Primers were referred from the Centers for Disease Control and Prevention website (https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html). The relative quantitative method was used for the quantitative analysis. Glyceraldehyde-3-phosphate dehydrogenase was used as an internal control to normalize samples. The expression level of each gene was calculated as $2^{-\Delta CT}$.

Formalin-fixed paraffin-embedded tissues were subjected to immunohistochemical analysis for endothelial Nitric Oxide Synthase (eNOS), a marker of endothelial function, (1:200; cat # ab76198; Abcam, Cambridge, UK). Immunohistochemistry images of eNOS staining were performed on a Leica Bond™ system using the standard protocol.

For fluorescent microscopy, paraffin sections of the penile tissue were incubated with COVID-19 spike protein, Rabbit pAb (1:100; cat# bs-0130R-Bioss; Novateinbio, Woburn, MA, USA) overnight at room temperature. Endothelial progenitor cell (EPC) function were assessed *ex vivo* by determination of endothelial colony forming units from blood samples collected from COVID-19 (+) and COVID-19 (-) men with severe ED.

RESULTS

The first specimen was collected from a patient status-post robotic assisted laparoscopic prostatectomy males 3 years prior. The patient's past medical history was significant for a 14-day hospitalization due to COVID-19. Other risk factors for ED such as hypertension,

coronary artery disease and diabetes mellitus were not present. The second specimen retrieved came from a patient with a past medical history significant for coronary artery disease and hypertension with a relatively mild case of COVID-19 (fever, cough, body aches) Both men had "normal erectile function" without the use of medications prior to their COVID infections.

TEM revealed extracellular viral particles ~100 nm in diameter, with prominent peplomers (spikes), and electron-dense dots of the nucleocapsid inside the particles near penile vascular endothelial cells of the COVID-19 (+) patients (Fig. 1). Notably, viral particles were not detected in tissue obtained from COVID-19 (-) men. There were no significant differences in H&E staining between COVID-19 (+) and COVID-19 (-) men. COVID-19 RNA was detected in both the penis biopsy samples from men with a history of COVID, but not in the samples from COVID-19 (-) men. Immunohistochemistry showed decreased eNOS expression in the corpus cavernosum of COVID-19 (+) men compared to COVID-19 (-) men, consistent with endothelial dysfunction (Fig. 2). COVID-19 spike protein–positive cells could not be detected by immunofluorescence despite positive COVID-19 PCR. EPC levels from the COVID-19 (+) men were 0 cell/well and 1.167 cell/well respectively compared to mean EPCs from 34 COVID-19 (-) men with severe ED (4.04 cells/well), suggesting impaired endothelial function.

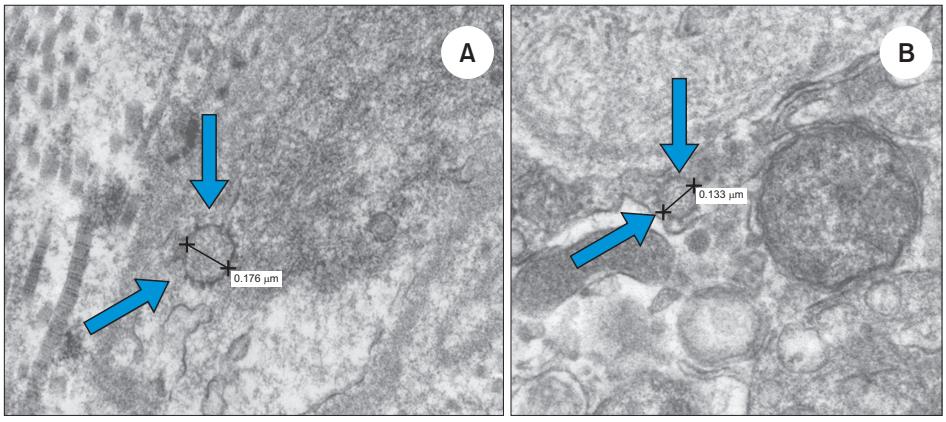

Fig. 1. Ultrastructure features of penile tissue from live seroconverted COVID-19 patients. (A) Coronavirus-like spiked viral particles (arrows) visualized *via* TEM in the peri-vascular erectile tissue of a live patient who had previously contracted the COVID-19 virus and subsequently seroconverted. Particle diameter measurement indicated on image. (B) Coronavirus-like spiked viral particles (arrows) visualized *via* TEM in the peri-vascular erectile tissue of a live patient who had previously contracted the Covid-19 virus and subsequently seroconverted. Particle diameter measurement indicated on image.

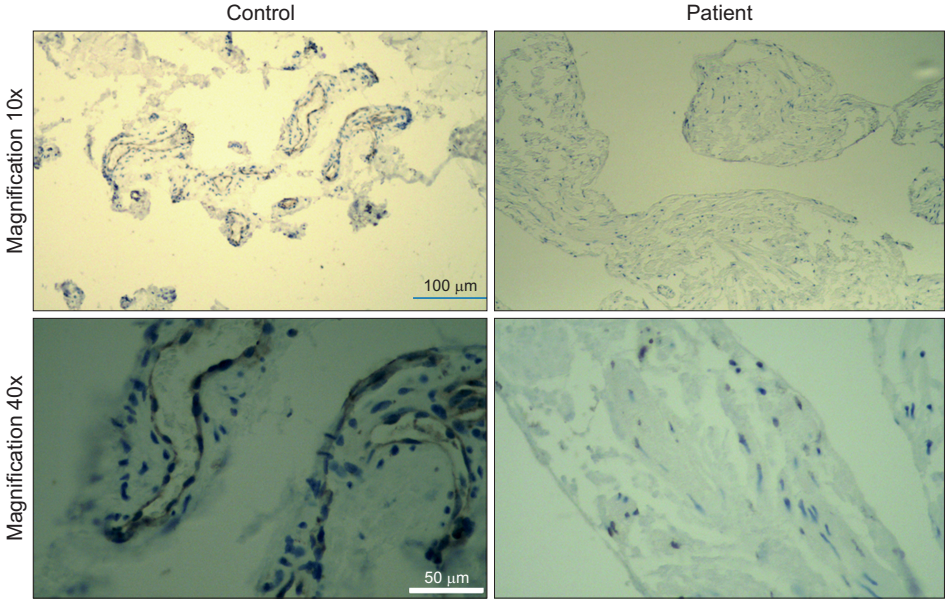

Fig. 2. Immunohistochemical comparison of endothelial nitric oxide synthase (eNOS) at both 40× and 10× magnification with the Leica Bond™ system using the standard protocol. Immunohistochemistry in the COVID-19 (-) patient (Control) had stained more intensely indicating relatively high expression of eNOS and normal endothelial function. The COVID-19 (+) patient (Patient) exhibited less intense staining indicating relatively low expression of eNOS which can indicate endothelial dysfunction and damaged vascular integrity.

DISCUSSION

In this report, we provide evidence of COVID-19 in the human penis long after the initial infection. Our study also suggests that endothelial dysfunction from COVID-19 infection can contribute to resultant ED. Vascular integrity is necessary for erectile function, and endothelial damage associated with COVID-19 is likely to affect the penile vascular flow, resulting in impaired erectile function. We could not detect viral protein in penis tissue by immunohistology, possibly due to comparably low viral RNA load in the penis. This result was not surprising since recent studies show that COVID-19 isolation is unlikely from samples with low RNA loads [4]. H&E staining from our specimens did not show significant results compared to previous studies in which COVID-19 infection caused mild to moderate accumulation of lymphomonocytic inflammatory cells in a perivascular or subendothelial distribution [5]. Based on the current findings we can draw two hypotheses about how the SARS-CoV-2 virus can lead to the ED. First, similar to other complications related to COVID-19, of ED can be the result of systemic infection resulting in widespread endothelial dysfunction. This is supported by our findings of endothelial dysfunction seen in men with COVID and ED. Second, we can also hypothesize that the worsening of these patient's ED can be due to the virus' presence within cavernosal endothelium itself. This is best supported by our findings with TEM. The primary limitation of this study were the sample size (n=2) and lack of objective quantification of erectile function before and after infection for patients and controls. For now, history of COVID-19 should be included in the work-up of ED and positive findings should be investigated accordingly. Patients should be aware of the potential complication of post-COVID-19 ED. Any changes observed in erectile function after infection should be followed up with the appropriate specialist for treatment and to help further investigation into the condition. Future studies are needed to validate the effects of this virus on sexual function.

CONCLUSION

This study is the first to demonstrate the presence of the COVID-19 virus in the penis long after the initial infection in humans. Our study also suggests that widespread endothelial cell dysfunction from COVID-19 infection can contribute to resultant erectile dysfunction. Future studies will evaluate novel molecular mechanisms of how COVID-19 infection can lead to ED.

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Conflict of Interest

The authors have nothing to disclose.

Author Contribution

Conceptualization: EK, JA, RR. Data curation: EI, HA, RS, JMH, FF, VWA. Formal analysis: HA. Funding acquisition: RR. Investigation: EK, JA, KK, HA. Methodology: EK, JA, RR. Project administration: RR. Resources: OK, HA. Supervision: RR. Validation: HA. Visualization: EK, JA. Writing – original draft: EK, JA. Writing – review & editing: EK, HA, RR, KK, RS.

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