Available online at www.medicinescience.org

Medicine Science International Medical Journal

ORIGINAL ARTICLE

Medicine Science 2022;11(4):1588-92

Cervicovaginal coronavirus disease 2019 (COVID-19) positivity: A pilot study

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Received 09 August 2022; Accepted 12 November 2022
Available online 27.11.2022 with doi: 10.5455/medscience.2022.08.181

Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can potentially infect female reproductive organs. In this study, we investigated the presence of SARS-CoV-2 in cervicovaginal fluid. This study included 31 female patients aged 18–65 years. The presence of SARS-CoV-2 RNA was investigated by RT-PCR in two separate cervicovaginal swab samples collected from patients 14 days apart. Viral RNA was extracted using Bio-Speedy ®vNAT® Viral Nucleic Acid Buffer (vNAT) solution, and SARS-CoV-2 RNA was analyzed using Bio-Speedy SARS-CoV-2 RT-qPCR kits in a Bio-Rad CFX96 Touch™ device. First and second cervical swab samples were collected 14 days apart. The SARS-CoV-2 RNA result was negative in all 53 cervicovaginal swab samples collected. Negative SARS-CoV-2 RNA results in cervicovaginal swab samples indicate that coronavirus disease 2019 (COVID-19) is not sexually transmitted. However, the number of studies on this subject and the sample size examined are still insufficient for reaching this conclusion.

Keywords: Cervicovaginal fluid, COVID-19, female genital system, sexual transmission

Introduction

Coronavirus disease 2019 (COVID-19), first appeared in December 2019 in a group of patients diagnosed with pneumonia of unknown origin in Wuhan, China [1]. The possible cause of this pneumonia was a new type of beta coronavirus. This new virus was identified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the disease it causes was named coronavirus disease 2019 (COVID-19) [2,3]. On March 11,2020, the WHO declared COVID-19 a pandemic when the number of SARS-CoV-2 cases outside of China increased 13-fold, with >118.000 cases and 4.000 deaths in 114 countries [4]. COVID-19 is primarily transmitted by droplets or direct contact [5,6]. In symptomatic patients, clinical symptoms comprising fever, cough, nasal congestion, fatigue, and other signs of upper respiratory tract infections usually begin in less than a week. Infection may progress to severe pneumonia with increasing shortness of breath and it can be fatal [7]. Although the course of COVID-19 is severe in the respiratory system, results are suggesting that it affects multiple organs outside of this system. In addition to lung damage, it can damage the heart, liver, kidneys, and nervous system [8-12]. The literature is limited on the possible consequences of SARS-CoV-2 infection in the male and female reproductive systems. Most studies have focused on the mechanisms leading to the development of COVID-19,

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possible treatments, and vaccines [13]. Autopsy studies indicate that SARS-CoV-2 is transmitted to the host via direct endothelial invasion using the angiotensin converting enzyme 2 (ACE 2) receptor in various organs, including the lung, heart, kidneys, and intestines [14,15]. ACE 2 RNA expression has also been detected in the prostate, vagina, fallopian tube, endometrium, and cervix. These suggest that SARS-CoV-2 could potentially infect all male and female reproductive tissues [13]. The presence of cervical coronavirus is of great importance in terms of sexual transmission, vertical transmission in pregnant women, and reproductive medicine. The study aimed to contribute to the scientific literature by investigating the presence of SARS-CoV-2 in cervicovaginal fluid.

Material and Methods

Patient selection and preparation of the samples

Our study included a total of 31 female patients aged 18-65 years (three of whom were pregnant) who applied to Ahi Evran University Education and Research Hospital between June 15 and September 1, 2020 and was positive for SARS-CoV-2 RNA based on nasopharyngeal swab samples. The presence of SARS-CoV-2 RNA was investigated by RT–PCR in two separate cervicovaginal swabs collected from the patients 14 days apart. Necessary permissions for the study were obtained from Ahi Evran University Faculty of Medicine Clinical Research Ethics Committee (Decision No: 2020-08/53) and the Ministry of Health (2020-04-30T01_35_51).

Collection of Cervicovaginal Fluid Samples

Informed consent was obtained from sexually active female patients whose diagnosis of COVID-19 was confirmed by nasopharyngeal SARS CoV-2 PCR test and their first-degree relative. Cervicovaginal swab samples were collected from the patients within two days of nasopharyngeal SARS CoV-2 PCR positivity. This was realized in the presence of auxiliary health personnel while wearing appropriate personal protective equipment (N95 mask, goggles, face protection, gloves, and overalls). The samples were collected from the cervical mouth, posterior fornix, and vaginal sidewalls by inserting a speculum while the patients were in the lithotomy position. Samples were appropriately placed in tubes containing Bio-Speedy vNAT.

SARS-CoV-2 RNA Analysis

The diagnosis of patients with suspected COVID-19 who applied to our hospital was conducted by the SARS CoV-2 Polymerase Chain Reaction (PCR) test. Viral RNAs extracted from the nasopharyngeal swab samples using viral nucleic acid buffer (vNAT) solution and were analyzed by Bio-Speedy SARS-CoV-2 RT–qPCR kits (single-step reverse transcription (RT) and real-time quantitative PCR (qPCR) kits targeting ORF1ab and N gene fragments [RT–qPCR]) and a Bio-Rad CFX96 TouchTM device. The cervicovaginal samples collected from the patients were appropriately transferred into tubes containing Bio-Speedy vNAT and then delivered to the microbiology laboratory as soon as possible. Viral RNAs extracted using vNAT solution were analyzed with Biospeedy SARS-CoV-2 RT–qPCR kits and a Bio-Rad CFX96 Touch™ device. The oligonucleotide set targeting the human RNase P gene (internal control) was examined for controlling sampling, nucleic acid extraction, and inhibition. The internal control (IC) (RNase P) curve is marked in blue, while the SARS-CoV-2 (N) and SARS-CoV-2 (ORF1ab) curves are marked in red. If the internal control curve is sigmoidal, this indicates that there is no problem regarding the sampling and isolation stages. Moreover, when the SARS-CoV-2 curve was sigmoidal, the sample was reported as POSITIVE (Figure 1), and when this curve was not sigmoidal, the sample was reported as NEGATIVE (Figures 2).

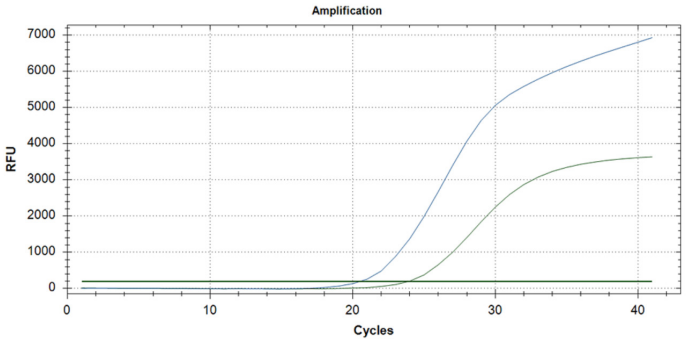

Figure 1. SARS-CoV-2 Positive Results

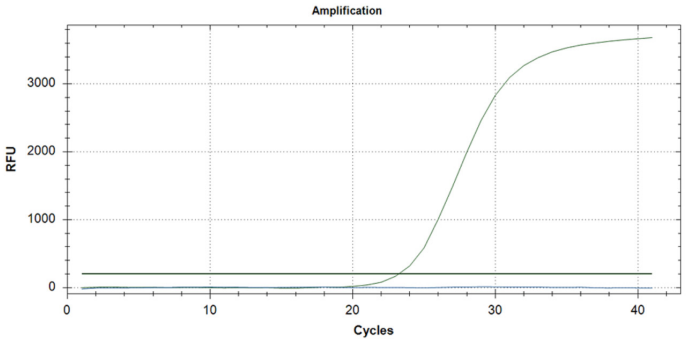

Figure 2. Negative SARS-CoV-2 Results Statistical analysis

SPSS software, version 22.0, was used for statistical analysis. Continuous values were presented as the mean (Standard Deviation) if they were normally distributed or the median (Inter Quartile Range) if they were not, and categorical variables are presented as counts (%). For laboratory results, we also assessed whether the measurements were outside the normal range.

Results

A total of 31 female patients aged 20–64 years were included in the study. The mean age of the patients was 46.097 (±13.207), mean gravida (number of pregnancies) was 2.677 (±1.720), and parity (number of live births) was 2.387 (±1.801). Note that 12 (38.7%) of the patients had regular menstruation, 16 (51.7%) were in menopause, and 3 (9.6%) were pregnant. The most common accompanying chronic diseases were hypertension (n=9, 29.0%), diabetes (n=4, 12.9%), and chronic pulmonary disease (n= 4, 12.9%). Only 11 (35.4%) patients had COVID-19-positive spouses. Moreover, 96.7% (n, 30) of the patients had muscle and joint pain, 93.5% (n, 29) had a weakness, and 54.8% (n, 17) had a cough. Note that 27 (87.1%) patients whose overall condition was good were followed up in the ward, and 4 (12.9%) patients whose

overall condition was moderate were followed up in the intensive care unit. There were no patients with poor overall conditions which required intubation (Table 1).

Table 1. Clinical findings

Symptoms	**N(%)**
Muscle and Joint Pain	30(%96.7)
Weakness	29(%93.5)
Cough	17(%54.8)
Loss of Taste	9(%29)
Loss of Smell	9(%29)
Shortness of Breath	8(%25.8)
Fever	5(%16.1)
Findings	**Mean(±SD)**
Fever	36.9(±0.68)
Saturation	91.9(±5.56)
Status	N (%)
Good	27(%87.1)
Moderate	4(%12.9)
Poor	0
Follow-up	**N (%)**
Ward	27(%87.1)
Intensive Care	4(%12.9)
Intensive Care (Intubation)	0
Treatment	**N (%)**
Hydroxychloroquine +Azithromycin +Enoxaparin	16(%51.6)
Hydroxychloroquine +Favipiravir +Enoxaparin	12(%38.7)
Lopinavir + Ritonavir + Enoxaparin	3(%9.7)

While 77.4% (n, 24) of patients had lung involvement on computerized tomography (CT) consistent with COVID-19, 12.9% (n, 4) had no lung involvement on CT. Mean laboratory results were as follows: White blood cell (WBC): 5.7 (2.0-14.3), c-reactive protein (CRP): 2.2 (0.2-10.9), ferritin: 86.7 (5.0-577.0), troponin I: 2.3 (0.2-9.3), D-dimer: 0.52 (0.34), and fibrinogen: 387.3 (255.0-624.0) (Table 2).

Table 2. Imaging and laboratory results

Thoracic Tomography	**N (%)**	
Lung Involvement (+)	24(%77.4)	
Lung Involvement (-)	9(%12.9)	
CT not available	3(%9.7)	
Laboratory	**Mean (Min-Max)**	
White Blood Cell	5.7(2.0-14.3)	10^3/µL
Hemoglobulin	12.7(7.8-16.8)	g/dL
Platelet	222(118-336)	10^3/µL
Glucose	105.9(61.0-292.0)	mg/dL
Urea	25.1(8.0-50.0)	mg/dL
Creatinine	0.6(0.31-1.17)	mg/dL
Aspartate Aminotransferase	26.6(13.0-42.0)	U/L
Alanine Aminotransferase	21.5(7.0-41.0)	U/L
C-Reactive Protein	2.2(0.2-10.9)	U/L
Ferritin	86.7 (5.0-577.0)	ml/ng
Troponin I	2.3(0.2-9.3)	ng/mL
D-Dimer	0.52(0.2-1.55)	ng/ml
Fibrinogen	387.3(255.0-624.0)	mg/dL

Table 3. Severe Acute Respiratory Syndrome Coronavirus 2 PCR Results

Patient No	**Nasopharyngeal PCR (Spouse's)**	**Nasopharyngeal PCR (Day 1)**	**Nasopharyngeal PCR (Day 14)**	**Cervicovaginal PCR (Day 1)**	**Cervicovaginal PCR (Day 14)**
1*	(+)	(+)	(-)	(-)	(-)
2	(+)	(+)	(-)	(-)	(-)
3	(+)	(+)	(-)	(-)	(-)
4	(+)	(+)	(-)	(-)	0
5	(-)	(+)	(-)	(-)	0
6	(+)	(+)	(-)	(-)	(-)
7	(+)	(+)	(-)	(-)	0
8	(-)	(+)	(-)	(-)	(-)
9*	(+)	(+)	(-)	(-)	(-)
10*	(-)	(+)	(-)	(-)	(-)
11	(+)	(+)	(-)	(-)	(-)
12	(-)	(+)	(-)	(-)	(-)
13	(+)	(+)	(-)	(-)	(-)
14	(-)	(+)	(-)	(-)	0
15	(-)	(+)	(-)	(-)	0
16	(-)	(+)	(-)	(-)	0
17	(-)	(+)	(-)	(-)	(-)
18	(-)	(+)	(-)	(-)	0
19	(-)	(+)	(-)	(-)	(-)
20	(-)	(+)	(-)	(-)	(-)
21	(+)	(+)	(-)	(-)	(-)
22	(-)	(+)	(-)	(-)	(-)
23	(-)	(+)	(-)	(-)	0
24	(-)	(+)	(-)	(-)	(-)
25	(-)	(+)	(+)	(-)	(-)
26	(-)	(+)	(+)	(-)	(-)
27	(-)	(+)	(+)	(-)	(-)
28	(-)	(+)	(-)	(-)	(-)
29	(-)	(+)	(-)	(-)	(-)
30	(-)	(+)	(-)	(-)	0
31	(+)	(+)	(+)	(-)	(-)

*: Pregnant, (+): PCR positive, (-): PCR negative, 0: PCR analysis not available

The patients were hospitalized for an average of 14 days for treatment and follow-up purposes. No deaths because of COVID-19 or any other cause were observed in the patients included in our study. Four patients in the intensive care unit were discharged with recovery after their treatment. One of the three pregnant women gave birth to a preterm baby by a normal vaginal route at 34 weeks of gestation; however, the other two pregnant women gave birth to term babies by cesarean section. SARS-CoV-2 RNA results were negative in nasopharyngeal swab samples of babies after birth. The preterm baby born at 34 weeks of gestation was followed up in the neonatal intensive care unit for ~1 week because of prematurity. No other problem was observed afterward. There was no evidence of COVID-19 in the one-month follow-up of babies. The first nasopharyngeal swab sample was SARS-CoV-2 RNA positive in all patients (n, 31). The control nasopharyngeal swab samples after 14 days were negative for SARS-CoV-2 RNA in 87.1% (n, 27) of the patients and positive in 12.9% (n, 4) of the patients. The first and second cervical swab samples were collected from 22 patients 14 days apart. However, for 9 patients even if the first cervical swab was collected from nine patients, the second swab could not be collected after 14 days because they were menstruating or missed the appropriate time for sampling. The SARS-CoV-2 RNA result was negative in all 53 cervicovaginal swab samples collected (Table 3).

Discussion

In this study, the presence of SARS-CoV-2 RNA in a total of 53 cervicovaginal fluid samples from 31 patients aged 18-65 years who were diagnosed with COVID-19 and had mild to moderate clinical symptoms was investigated by RT–qPCR. The presence of SARS-CoV-2 RNA could not be demonstrated in any of the samples taken. This study showed us that SARS-CoV-2 RNA is not at a level that can be detected in the cervix and vagina, which is the female lower genital tract.

2019 novel coronavirus (2019-nCoV, also known as SARS-CoV-2), a beta coronavirus, is the seventh member of the coronavirus family that infects humans after MERS-nCoV and SARS-nCoV [16]. It binds to angiotensin-converting enzyme (ACE) 2 via the surface spike protein, which infects the target cell and causes severe damage similar to SARS-CoV [17,18]. ACE2 RNA expression is reported in all reproductive tissues of women (vagina, ovaries, fallopian tubes, endometrium, and cervix) and men (ductus deferens, testis, epididymis, seminal vesicle, and prostate) [13]. The study by Li et al. demonstrated that the presence of SARS-CoV-2 may be detected in semen during the active phase of the disease [19]. This suggests that SARS-CoV-2 can be sexually transmitted. Moreover, it has the potential to infect female reproductive tissues despite low susceptibility to SARS-CoV-2 infection [13]. The first study investigating the presence of SARS-CoV-2 RNA in the cervicovaginal fluid was conducted by Qiu et al. (16). This study was conducted on ten postmenopausal women aged 52–80 years who were hospitalized with a diagnosis of severe COVID-19. Vaginal swab samples were collected from patients 17–40 days after the onset of SARS-CoV-2 infection, and they were analyzed for SARS-CoV-2 RNA. The SARS-CoV-2 result was negative in all RT–PCR tests. Note that SARS-CoV-2 may have transmission mechanisms similar to SARS-CoV, but no report indicated that SARS-CoV invaded the female reproductive system, or no reports on the detection of SARS-CoV in vaginal fluid. Moreover, the authors noted that the absence of SARS-CoV-2 in the vaginal fluid was proof that there was no sexual transmission or vertical transmission from mother to baby [16]. In one of the first studies on this subject, Cui et al. investigated SARS-CoV-2 RNA in cervicovaginal and anal swab samples of 35 patients diagnosed with COVID-19. Of all the samples, SARS-CoV-2 RNA was detected in only one anal swab sample. All other samples were negative for SARS-CoV-2 RNA. RT–PCR positivity could not be observed in the vaginal environment because of the absence of ACE2 expression, which is the receptor SARS-CoV-2 binds, in the tissues of the vagina and cervix. The authors noted that there was no evidence that SARS-CoV-2 was transmitted from a woman to her partner via vaginal intercourse [17]. In addition, SARS-CoV-2 was detected in the cervicovaginal region only in a few studies [20,21].

Aslan et al. investigated the presence of SARS-CoV-2 RNA in the vaginal fluid of pregnant women with COVID-19. Twelve pregnant women with mild symptoms of confirmed COVID-19 were included in the study, and the presence of SARS-CoV-2 RNA was investigated in vaginal swab samples. All samples tested negative for SARS-CoV-2 RNA [18]. However, in a systematic review including 156 newborns, the vertical transmission rate was 3.91% [22]. Despite the increasing number of published studies on COVID-19 in pregnancy, the data is insufficient to draw unbiased conclusions about the complications of COVID-19, vertical transmission, and perinatal complications in pregnant women.

In this study, the first samples were collected from patients during the active disease period. The second samples were collected after 14 days. Nevertheless, all samples were negative for SARS-CoV-2 RNA, which was consistent with the literature. When the perinatal results of three pregnant women in the study were evaluated, the SARS-CoV-2 RNA result was negative based on the nasopharyngeal swabs of the three newborns. In terms of health status, there were no results suggestive of COVID-19. The main difference of this study from other studies was that sequential samples were collected during the active disease period. Moreover, the number of patients, age range, and inclusion of pregnant patients increased the observation power of the study, and the last of these also allowed probing vertical transmission.

Although the number of patients in this study is comparable to the sample size of similar studies, it is still one of its primary limitations. Another limitation is that the study was conducted in a single center. Studies on COVID-19 are rapidly continuing, and there is a requirement for comprehensive multi-center studies that include more cases.

Conclusion

Negative SARS-CoV-2 RNA results in cervicovaginal swabs indicate that there is no evidence suggesting that COVID-19 is sexually transmitted. However, the number of studies on this subject and the sample size examined are still not sufficient. More comprehensive studies are needed on this subject.

Conflict of interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

The authors declare that they have received no financial support for the study.

Ethical approval

Ethics committee approval was obtained from the Ahi Evran University Ethics Committee (Date: June 10, 2020 Number: 2020-08/53).

References

1. Wiersinga WJ, Rhodes A, Cheng AC, et al. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): A review. JAMA. 2020;324:782-93.
2. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in china, 2019. N Engl J Med. 2020;382:727-33
3. Liu Y, Gayle AA, Wilder-Smith A, Rocklöv J. The reproductive number of COVID-19 is higher compared to SARS coronavirus. J Travel Med. 2020;27:taaa021.
4. Velavan TP, Meyer CG. The COVID-19 epidemic. Trop Med Int Health. 2020;25:278-80.
5. World Health Organization. Coronavirus disease 2019 (COVID-19): situation report, 72.2020
6. World Health Organization. Modes of transmission of virus causing COVID-19: implications for IPC precaution recommendations: scientific brief, 27.4.2020
7. Chan JF, Yuan S, Kok KH, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet. 2020;395:514-23.
8. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395:497-506.
9. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA. 2020;323:1061-9.
10. Zhang C, Shi L, Wang FS. Liver injury in COVID-19: management and challenges. Lancet Gastroenterol Hepatol. 2020;5:428-30.
11. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020;395:507-13.
12. Mao L, Jin H, Wang M, et al. Neurologic manifestations of hospitalized patients with coronavirus disease 2019 in wuhan, china. JAMA Neurol. 2020;77:683-90.
13. Zupin L, Pascolo L, Zito G, et al. SARS-CoV-2 and the next generations: which impact on reproductive tissues?. J Assist Reprod Genet. 2020;37:2399-403.
14. Varga Z, Flammer AJ, Steiger P, et al. Endothelial cell infection and endotheliitis in COVID-19. Lancet. 2020;395:1417-8.
15. Ackermann M, Verleden SE, Kuehnel M, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. N Engl J Med. 2020;383:120-8.
16. Qiu L, Liu X, Xiao M, et al. SARS-CoV-2 Is not detectable in the vaginal fluid of women with severe COVID-19 ınfection. Clin Infect Dis. 2020;71:813-817.
17. Cui P, Chen Z, Wang T, et al. Clinical features and sexual transmission potential of SARS-CoV-2 infected female patients: a descriptive study in Wuhan, China. MedRxiv. 2020; doi: https://doi.org/10.1101/2020.02.26.20028225
18. Aslan MM, Uslu Yuvacı H, Köse O, et al. SARS-CoV-2 is not present in the vaginal fluid of pregnant women with COVID-19. J Matern Fetal Neonatal Med. 2022;35:2876-8.
19. Li D, Jin M, Bao P, et al. Clinical characteristics and results of semen tests among men with coronavirus disease 2019. JAMA Netw Open. 2020;3:e208292.
20. Khoiwal K, Kalita D, Shankar R, et al. Identification of SARS-CoV-2 in the vaginal fluid and cervical exfoliated cells of women with active COVID-19 infection: A pilot study. Int J Gynaecol Obstet. 2021;153:551-3.
21. Schwartz A, Yogev Y, Zilberman A, et al. Detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in vaginal swabs of women with acute SARS-CoV-2 infection: a prospective study. BJOG. 2021;128:97-100.
22. Chi J, Gong W, Gao Q. Clinical characteristics and outcomes of pregnant women with COVID-19 and the risk of vertical transmission: a systematic review. Arch Gynecol Obstet. 2021;303:337-45.