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Original Article

Investigation of SARS-CoV-2 using RT-PCR in vaginal swab samples of female patients with a diagnosis of severe COVID-19

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ABSTRACT

Objective: It is important to determine the presence of SARS-CoV-2 in the vaginal fluid samples of reproductive-aged women with severe disease during the acute stage of the disease and to determine the risks of transmission by sexual or vertical transmission.

Material and methods: Adult women with confirmed severe COVID-19 who were admitted to Ankara City Hospital intensive care unit (ICU) between December 1st, 2020, and January 1st, 2021, were enrolled in the study. Vaginal swab samples were collected within 48 h in the ICU using Dacron or rayon swabs and tested for SARS-CoV-2 using reverse transcription real-time polymerase chain reaction (RT PCR).

Results: Thirty women of reproductive age were included in the study, five (16.7%) of whom were pregnant. The mean age was 44.9 (±10.5) years. The most common symptoms were headache (100%), muscle soreness (86.7%), cough (76.7%), fever (60%), and nausea and vomiting (20%). Nineteen (63.3%) patients had underlying medical conditions. The time interval from obtaining vaginal swab samples to admission to the ICU was 48 h. The time between vaginal sampling and PCR positivity ranged from 2 to 18 days. SARS-CoV-2 was not detected in any vaginal samples.

Conclusion: Our study showed that women with severe COVID-19 did not have SARS-CoV-2 in their vaginal fluids. Investigation of the presence of SARS-CoV-2 in vaginal secretions may help in determining the risks of sexual transmission and vertical transmission from mother to baby. Information on this subject is still limited. Larger studies on comprehensive biological samples are needed.

Introduction

In late 2019, many cases of severe pneumonia that was accompanied by cough and/or fever resulting in sudden deaths were reported in Wuhan City, China. Following further investigations and genomic sequencing of clinical samples from the respiratory tracts of infected individuals, the pathogen was identified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the disease was called coronavirus 2019 (COVID-19) by the World Health Organisation (WHO) [1,2]. As of April 8th, 2022, there were more than 14 million confirmed cases in Turkey and 494 million cases worldwide [3].

The sudden outbreak of COVID-19 infections, together with its highly contagious nature, led scientific researchers to take immediate action on preventing the clinical deterioration of the disease and to gain a better understanding of the basic mechanism of transmission pathways [4]. SARS-CoV-2 is primarily transmitted person-to-person through respiratory droplets and aerosols from both symptomatic individuals and asymptomatic carriers shedding the virus while talking, coughing, and sneezing. Prolonged exposure in close contact with a person carrying a high virus load may also cause infection [5,6].

Angiotensin-converting enzyme 2 (ACE2) plays a crucial role in the course of COVID-19 disease pathogenesis. SARS-CoV-2 has a

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high binding affinity to ACE2, and the virus can enter the target host cells by using ACE2 as a receptor for the receptor-binding domain (RBD) of its spike (S) protein [5,7]. Another important structure that participates in the mechanism of entry of SARS-CoV-2 into target cells is transmembrane serine protease 2 (TMPRSS2), which is the major cofactor of ACE2 [8].

Besides the type II alveolar cells of the lungs, ACE2 can also be expressed by different tissues and organs such as the heart, esophagus, kidneys, gastrointestinal tract, bladder, and testis. Previous studies also identified ACE2 in the ovaries [9], uterus, and vagina [10]. TMPRSS2 expression is similar to ACE2 expression in many tissues such as the lungs and type II alveolar cells, kidney, liver, bladder, gastrointestinal tract, and testis [4,11,12]. Despite SARS-CoV-2 primarily affecting the respiratory system and particularly the lungs, the wide expression of ACE2 and TMPRSS2 in different human tissues suggests that the virus might use alternative pathways, and these tissues could also be more vulnerable to SARS-CoV-2 infection [13,14].

Previous studies have shown that SARS-CoV-2 was present in bodily fluids such as feces, blood, serum, and urine [15,16]. From a limited number of studies investigating the presence of SARS-CoV-2 in semen samples, only one study detected the virus in semen [17]. Although this finding raised concerns about possible viral shedding in the human reproductive system, the outcomes of recent studies identifying the presence of SARS-CoV-2 in vaginal specimens manifested contradictory results. This study aimed to determine whether SARS-CoV-2 was present in the vaginal fluid of women with severe COVID-19.

Materials and methods

Study design and participants

This study was designed as a prospective cross-sectional study that was conducted in Ankara City Hospital. Women aged 24–56 years with a confirmed diagnosis of severe COVID-19 who were followed in the Anesthesiology and Reanimation Intensive Care Unit (ICU) between December 1st, 2020, and January 1st, 2021, and agreed to participate were enrolled in the study. This study was approved by the Local Ethics Committee 1 of Ankara City Hospital (Date: 21st Nov 2020/Decision no: E1/1306/2020). Written informed consent was obtained from all participants before their inclusion in the study. Informed consent forms were obtained from themselves in conscious patients, and from first-degree relatives in intubated patients.

Exclusion criteria

Patients who are not in reproductive age, those with negative nasal/oropharyngeal PCR samples, and those who did not agree to participate in the study were excluded from the study.

Laboratory examinations

Nasal and oropharyngeal samples were analysed using reverse transcription real-time polymerase chain reaction (RT PCR) testing for SARS-CoV-2.

Laboratory tests performed on the day of referral to the ICU included haematology (white blood cell [WBC], haemoglobin, lymphocyte, neutrophil, and platelet [PLT] counts) and biochemistry (urea, creatinine, alanine aminotransferase [ALT], aspartate aminotransferase [AST], lactate dehydrogenase [LDH], total bilirubin), cardiac enzymes (N-terminal prohormone of brain natriuretic peptide [NT-proBNP], troponin I), coagulation parameters (international normalised ratio [INR], D-dimer, fibrinogen), acute-phase reactants (ferritin, C-reactive protein [CRP], procalcitonin [PCT], interleukin-6 [IL6]), and the Acute Physiology and Chronic Health Evaluation II (APACHE II) scoring.

Vaginal sample collection

Vaginal fluid samples were taken within 48 h of ICU admission. Sterile Dacron or rayon swabs with a flexible plastic shaft were used to take a sample of vaginal fluid. Swabs were inserted approximately 2 cm into the vagina and rotated for 3–5 s. Swabs were then placed into 2 mL sterile Viral Transport Medium (VTMs from several manufacturers), and immediately transferred to Ankara Numune Hospital Molecular Microbiology Laboratory to be analysed.

Nucleic acid isolation

SARS-CoV-2 detection in vaginal swab samples was performed using RT-PCR for SARS-CoV-2-specific 'Orf1ab' and 'N' genes targeting human Ribonuclease P (RNaseP) genes. RNaseP was used as an internal control to evaluate sample-based inhibition control and kit reagent control.

RNA extraction from vaginal swab samples was performed on a BioRobot EZ1 Advanced XL® system device (Qiagen, Hilden, Germany) using the EZ1 Virus Mini Kit (Qiagen, Hilden, Germany) according to the method recommended by the manufacturer.

Real-time RT PCR method

Real-time RT-PCR was performed using a Coronex COVID-19 (Version 2.0) Multiplex RT-qPCR Detection Kit (DS Bio and Nano Technology, Ankara, Turkey). A 20-μL reaction mix consisted of 5 μL of RNA, 12.5 μL of CORONEX-COVID-19 DS Mix E (RT-qPCR master mix), and 2.5 μL of CORONEX-COVID-19 DS PP1 primer-probe mix (Orf1ab and N genes for SARS-CoV-2 detection, and RNaseP gene for internal control). A positive control for amplification control and negative control to assess contamination were used. For reverse transcription, thermal cycling was performed at 48 °C for 20 min, followed by 95 °C for 5 min, and then 35 cycles at 95 °C for 5 s and 60 °C for 10 s in a Rotor-Gene Q device (Qiagen, Hilden, Germany). Amplification curves obtained after completion of the amplification process were evaluated, and cycle threshold (Ct) values less than 33 were defined as positive.

Statistical analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows 24.0 program. This was a descriptive study, and no formal sample size calculation was performed. Continuous variables are described as the mean, standard deviation (SD), minimum and maximum, and categorical variables are expressed in numbers and percentages (%).

Results

A total of 30 women aged between 24 and 56 years who tested positive for SARS-CoV-2 were enrolled in the study, 5 (16.7%) of whom were pregnant. The mean (±standard deviation [SD]) age of the patients was 44.9 (±10.5) years. One patient was postpartum, and two patients had undergone a cesarean delivery in the ICU unit. The patients were admitted to Ankara City Hospital due to symptoms including headache (100% of the patients), muscle soreness (86.7%) cough (76.7%), fever (60%), and nausea and vomiting (20%), and then transferred to the ICU with the diagnosis of severe COVID-19 infection. Thirteen (43.3%) patients needed mechanical ventilation during the study period. Of the patients, 17 (66.7%) received

oxygen support by nasal route or balloon mask, with or without oxygen support by high-flow nasal cannula. Nineteen (63.3%) patients had underlying medical conditions: hypertension (17.4%), asthma (15.2%), diabetes mellitus (10.9%), and hypothyroid (6.5%). Five patients died of severe COVID-19, and 14 were discharged from the hospital. The patient demographics and clinical characteristics are included in Table 1.

Twenty-three (76.7%) patients developed lymphopenia, and 13 (43.3%) had leukocytosis. Concentrations of D-dimer, CRP, PCT, and IL-6 were elevated in 26 (86.7%), 21 (70.0%), 16 (55.2%), and 28 (93.3) patients, respectively. Laboratory findings are presented in Table 2.

During their stay in the intensive care unit, the patients received steroid, antiviral therapy (except for pregnant women) and low molecular weight heparin therapy. These are listed in Table 3.

The time intervals from obtaining vaginal swab samples to the disease diagnosis and admission to the ICU were 2–18 days and 48 h, respectively. The samples were analysed for the presence of SARS-CoV-2 using RT-PCR. All vaginal sample tests were negative for SARS-CoV-2.

Discussion

In this study, we aimed to determine whether the presence of SARS-CoV-2 could be detected in the vaginal fluid of 30 women aged between 24 and 56 years hospitalised in the ICU with a clinical diagnosis of severe COVID-19. Vaginal swab samples were taken within 48 h after admission to the ICU. Samples were analysed using RT-PCR and all were negative for SARS-CoV-2. To the best of our knowledge, limited published reports exist in the literature investigating the virus in vaginal secretions to date, with conflicting results. Our findings are in line with some studies available to this point [18–22].

Recently, Milbak et al. [23] conducted a study with pregnant patients and found that 2 of 28 vaginal swab samples were positive for SARS-CoV-2. Vaginal samples were obtained from one patient in the intrapartum period and one in the antepartum period. Similar findings were demonstrated in a study of 31 pregnant women presenting mild and severe COVID-19 symptoms [24], and in a case report of a 23-year-old pregnant woman [22]. In one patient with a positive vaginal swab for SARS-CoV-2, samples from maternal plasma, the placenta, umbilical cord, and a nasopharyngeal swab of the neonate were also found positive [24]. Furthermore, SARS-CoV-2-specific IgG positivity was confirmed in a total of 19 maternal and 12 umbilical cord plasma samples. The key message in this research is that even though the presence of SARS-CoV-2 was not detected in vaginal specimens of infected mothers, vertical transmission is possible from mothers to newborns, and immediate viral testing and close monitoring should be taken into consideration.

In concordance with our results, Aslan et al. [21] did not detect SARS-CoV-2 in RT-PCR tests in the vaginal fluids of 20 pregnant

Table 1
Demographic and clinical characteristics.

Variable	n = 30
Age (years)	
Mean (SD)	44.9 (10.5)
Min-max	24–56
Pregnancy status, n (%)	
Non-pregnant	25 (83.3)
Pregnant	5 (16.7)
Disease severity, n (%)	
Severe	30 (100.0)
Presenting symptoms, n (%)	
Headache	30 (100.0)
Muscle soreness	26 (86.7)
Cough	23 (76.7)
Fever	18 (60.0)
Underlying medical disorders, n (%)	
Hypertension	8 (17.4)
Asthma	7 (15.2)
Diabetes mellitus	5 (10.9)
Hypothyroid	3 (6.5)
Rhythm disorder	2 (4.3)
Aortic valve replacement	1 (2.2)
Respiratory support, n (%)	
Mechanical ventilation	13 (43.3)
Oxygen support by nazal cannula, ballon mask, and/or high flow nasal cannula	17 (66.7)
Clinical outcomes, n (%)	
Discharged	14 (46.6)
Remain in hospital	11 (36.7)
Died	5 (16.7)

Table 2
Main laboratory findings.

Parameter (Unit, Normal range)	n = 30, (%)
Blood cell counts	
White blood cell [WBC] ($\times 10^9$/L, 3.6–10.5)	16 (53.3)
Decreased	1 (3.3)
Increased	13 (43.3)
Lymphocytes ($\times 10^9$/L, 1.1–4)	7 (23.3)
Decreased	23 (76.7)
Neutrophils ($\times 10^9$/L, 1.5–7.7)	11 (36.7)
Increased	19 (63.3)
Haemoglobin (12–18 g/dL)	14 (46.7)
Decreased	16 (53.3)
Platelets ($\times 10^9$/L, 160–400)	24 (80.0)
Decreased	3 (10.0)
Increased	3 (10.0)
Blood biochemistry	
Urea (19–49 mg/dL)	21 (70.0)
Decreased	4 (13.3)
Increased	5 (16.7)
Creatinine (0.7–1.3 mg/dL)	9 (30.0)
Decreased	19 (63.3)
Increased	2 (6.7)
Alanine aminotransferase [ALT] (<50 U/L)	24 (80.0)
Increased	6 (20.0)
Aspartate transaminase [AST] (<35 U/L)	8 (26.7)
Increased	22 (73.3)
Lactate dehydrogenase [LDH] (120–246 U/L)	2 (6.7)
Increased	28 (93.3)
Total bilirubin (0.2–1.1 mg/dL)	28 (93.3)
Increased	2 (6.7)
Cardiac enzymes	
NT-PRO BNP (<450 ng/L)	14 (46.7)
Increased	16 (53.3)
Troponin I (<45 ng/L)	25 (83.3)
Increased	5 (16.7)
Coagulation parameters	
INR (0.8–1.2 INR)	24 (80.0)
Increased	6 (20.0)
D-Dimer (<0.55 mg/L)	3 (13.3)
Increased	26 (86.7)
Fibrinogen (1.7–4.2 g/L)	1 (3.3)
Increased	29 (96.7)
Acute phase reactants	
Ferritin (22–322 μg/L)	16 (53.3)
Decreased	1 (3.3)
Increased	13 (43.3)
C-reactive protein concentration [CRP] (0–0.005 g/L)	9 (30.0)
Increased	21 (70.0)
Procalcitonin [PCT] (<0.16 μg/L)	13 (44.8)
Increased	16 (55.2)
Interleukin 6 [IL-6] (0–3.4 pg/mL)	2 (6.7)
Increased	28 (93.3)
APACHE II, mean (SD)	12.9 (8.0)

APACHE II: the Acute Physiology and Chronic Health Evaluation II, INR: International normalized ratio, NT-PRO BNP: N-terminal prohormone BNP.

Table 3
Applied drug treatments on patients.

Drugs used in treatment (Number of Patients)	n = 30, (%)
Steroids	30 (100)
Low-molecular-weight-heparin (LMWH)	30 (100)
Anti-viral therapy	25 (83.3)

women with moderate symptoms of COVID-19 who did not require hospital care. In another study, Yuvaci et al. [25] analysed vaginal swabs for SARS-CoV-2 in 18 women of reproductive age and could not demonstrate the presence of the virus in any samples using RT-PCR. Moreover, in a similar study investigating vaginal samples from 23 pregnant and eight non-pregnant women, there was no virus present in the samples taken [19]. These results are supported by a previous study of 38 patients aged 27–45 years with negative vaginal RT-PCR tests for SARS-CoV-2 [18].

Cui et al. [26, 27] conducted a study in a more heterogeneous population consisting of 28 menopausal, six premenopausal, and one postpartum woman, most of whom (91.4%) had severe symptoms. The authors reported that the samples taken from the vaginal fluid in all patients tested negative for SARS-CoV-2, and only one anal swab sample was found to be positive. However, unlike our study, eight patients who had negative nasopharyngeal RT-PCR test results were included in this study based on their positive chest computed tomography (CT) findings and disease symptoms. Another difference from our study was that two separate vaginal fluid samples were obtained from each individual and tested in different laboratories to avoid false negatives.

In a recent study involving 52 pregnant women with mild-to-moderate COVID-19 symptoms [28], umbilical cord, placental, amniotic, and vaginal fluid samples were collected from all patients. SARS-CoV-2 was not detected in amniotic fluid and umbilical cord samples, but one placental and two vaginal samples tested positive for the virus. In this study, nasopharyngeal swabs and blood samples were also obtained from the newborns to screen for SARS-CoV-2. The nasopharyngeal swab of one newborn was reported as PCR-positive for the virus, and two newborns were both virus-specific IgG and IgM positive. Surprisingly, their mothers had negative results for all biologic samples. Even though there is insufficient evidence on the possibility of vertical transmission, there is a need for further research on the risk of mother-to-infant infection.

Similarly, Khoiwal et al. [29] examined the presence of SARS-CoV-2 in 15 vaginal and 12 cervical samples taken from 15 pre- and post-menopausal women. It is also worth mentioning that all samples were analysed using both RT-PCR and transcription-mediated amplification (TMA) assay, which is an approved technique by the United States of America Food and Drug Administration (FDA) for emergency use. All vaginal samples tested negative in RT-PCR but three samples were positive in TMA. All cervical samples were found negative with both techniques. This study highlighted the importance of using different methodologies in the detection of SARS-CoV-2 in human specimens. A study by Gorzalski et al. revealed that TMA assays had greater sensitivity than RT-PCR for identifying the virus in nasopharyngeal swabs [30].

In the study of Schwartz et al., vaginal swabs of two patients, one in reproductive age and the other postmenopausal, were found positive. Although these results are not compatible with our study, the clinician should consider the possibility of vaginal colonization, especially at birth [31].

The strengths of our study are its prospective nature and homogenous patient population. All women included in the study were of reproductive age and had a positive nasopharyngeal RT-PCR result for SARS-COV-2. Additionally, all patients presented with severe symptoms requiring care and follow-up in the ICU.

The present study also has several potential limitations. Although the sample size is larger than in some previous studies in the literature, it can be considered a limitation. Even though vaginal samples were obtained 48 h after the ICU admission, the time interval between sampling and the date of disease confirmation was 2–18 days. These data revealed that the samples were not collected in the early stage of the acute infection. After a certain time during the hospitalisation, additional sampling could have been performed to evaluate whether the course of the disease would affect the presence of the virus in vaginal specimens.

Conclusion

In our study, the presence of SARS-CoV-2 could not be detected in women of reproductive age with severe COVID-19 infection. When considered together with the results of the above-mentioned studies, the risk of vaginal contamination with SARS-CoV-2 can be considered as low. However, there are no long-term studies investigating the presence of the virus and its effects on the female reproductive system. Further studies with larger cohorts should focus on this subject. Nevertheless, it is too early to conclude that sexual and vertical transmission of SARS-CoV-2 may be excluded.

Author contributions

Concept – D.E.; **Design** – D.E., B.K., E.Y.Ç.; **Supervision** – D.E., B.D., I.Ö.T., R.G.; **Fundings** – E.Y.Ç., D.A.; **Materials** – E.Y.Ç., F.K.; **Data collection and/or processing** – D.E., B.K., E.Y.Ç., B.D., D.A., F.K., I.Ö.T., R.G., **Analysis and/or interpretation** – D.E., B.K., B.D.; **Literature search** – D.E., E.Y.Ç., F.S. **Writing** – D.E.; **Critical review** – D.A., B.K., E.Y.Ç., I.Ö.T., R.G.

Data availability statement

Data may be made on reasonable request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions. OR not applicable.

Funding statement

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Ethics statement

This study has been approved by the Local Ethics Committee of Ankara City Hospital and in accordance with the ethical standards of the Declaration of Helsinki.

Patient consent statement

Written informed consent was obtained from all participants.

Declaration of competing interest

The authors declare no conflict of interests.

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