

Detection of severe acute respiratory syndrome corona virus 2 in cervico-vaginal secretion of COVID-19-affected female: A prospective observational study from India

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Mukta Agarwal¹ , Swmkwr Basumatary¹,
Divendu Bhusan² and Binod Kumar Pati³

Abstract

Background: The novel severe acute respiratory syndrome corona virus 2 (also known as 2019-nCoV) is a highly infectious agent and is declared as a global public health emergency by the World Health Organisation. The main known transmission route of severe acute respiratory syndrome corona virus 2 is through respiratory air droplets. Although recent studies have revealed that the virus is detectable in the throat, blood, urine, anal swabs, tears and even faeces; however, modes of transmission other than respiratory droplets has not been studied much. Knowledge on the presence of the virus in the female genital tract may help determine the risk of sexual transmission as well as the risk of mother-to-child transmission. However, not much data are available yet regarding the presence of the virus in the female genital system. Hence, to explore the presence of the virus in the female genital system and possibility of sexual transmission, a study was conducted where in we tried to detect severe acute respiratory syndrome corona virus 2 in cervico-vaginal secretions.

Methods: From July 2020 to September 2020, 35 COVID-19-positive female patients admitted to tertiary care teaching institute of Eastern India, which is now declared dedicated Corona Hospital and Centre of Excellence for COVID-19 care, who consented for the research were enrolled in this prospective observational study. Proper gynaecological history, clinical records along with laboratory findings of the patient was recorded. The possibility of the sexual transmission of the virus from female to her male partner was to be ascertained by testing the presence of severe acute respiratory syndrome corona virus 2 in the vaginal, cervical secretions by reverse transcriptase polymerase chain reaction.

Results: All 35 COVID-19-positive female patients were tested for severe acute respiratory syndrome corona virus 2 in their vaginal and cervical secretions by reverse transcriptase polymerase chain reaction. All the samples were tested negative for the virus.

Conclusion: Findings from this study reveals that severe acute respiratory syndrome corona virus 2 is not present in the cervical and vaginal secretions, and the possibility of transmission from female to her male partner by vaginal sexual intercourse is unlikely.

Keywords

COVID-19, sexual transmission, vaginal secretions, reverse transcriptase polymerase chain reaction, vaginal swab

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Introduction

The novel severe acute respiratory syndrome (SARS) corona virus 2 (SARS-CoV-2; also known as 2019-nCoV) has played havoc worldwide, beginning with Wuhan, China in December 2019.¹ The main route of transmission is through respiratory droplet infection, but there is ongoing research to look into other possible routes. In order to effectively block its transmission, it is urgent to uncover all the possible transmission routes of SARS-CoV-2.

¹Obstetrics and Gynaecology, All India Institute of Medical Sciences Patna (AIIMS Patna), Patna, India

²Medicine, All India Institute of Medical Sciences Patna (AIIMS Patna), Patna, India

³Microbiology, All India Institute of Medical Sciences Patna (AIIMS Patna), Patna, India

Corresponding author:

Mukta Agarwal, Obstetrics and Gynaecology, All India Institute of Medical Sciences Patna (AIIMS Patna), Phulwari Sharif, Patna 801507, India.

Email: drmuktaa@aiimspatna.org



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The transmission of different corona viruses pandemics in the past mainly occurred via respiratory droplets; however, possibility of other modes of transmission were not explored much although presence of the virus were established in blood, urine, anal swabs, saliva, tears and even faeces. For example, SARS-COV which emerged in Guangdong province, mainland China in November 2002 was demonstrated by reverse transcriptase polymerase chain reaction (RT-PCR) to be present in faces, urine and tissue specimen from lung biopsy indicating that the infection is not confined to the respiratory tract.² Its primary mode of transmission appears to be through direct or indirect contact of mucous membrane of eyes, nose or mouth with infectious respiratory droplets or fomites. The role of faeco-oral transmission of SARS-CoV is unknown but may be important, given the fact that profuse watery diarrhoea is a common feature of the disease and that the virus is shed in large quantities in stool.³ Also no existing data exist to show if SARS-CoV can be sexually transmitted. Another corona virus, Middle East respiratory syndrome-related corona virus (MERS COV) which originated in April 2012, Jeddah in Saudi Arabia, were transmitted mainly by large respiratory droplets, and its transmission via sexual route is not clear.⁴ To study on mode of transmission of HCOV 229E, another corona virus, vaginal swabs and maternal nasal aspirates were performed at the beginning of the labour. Also, newborn gastric and rhinopharyngeal secretions were also collected. Vaginal swabs and new born gastric aspirate tested positive for the virus.⁵ Also, there is possibility of sexual transmission of HCOV 229E although not proven.

Studies have shown SARS-CoV-2 to be present in blood, urine, anal swabs, tears and even in the faeces.⁵ Not much data are available regarding the presence of the virus in the female genital system, which gives an opportunity to study its presence and hence the possible transmission via sexual route. Some studies have suggested the presence of SARS-CoV in the testis of male patients but not in the ovaries or uterus.⁶ However, the same has not been studied for SARS-CoV-2 yet. Keeping in mind these facts, we aim to check for the presence of SARS-CoV-2 virus in vaginal environment of COVID-19-affected patients, which might help determine the risk of sexual transmission as well as the risk of mother-to-child transmission.

Methods

Study design and setting

Prospective observational study, conducted from July 2020 to September 2020, in a tertiary care teaching institute of Eastern India, which is now declared dedicated Corona Hospital and Centre of Excellence for COVID-19 care.

Participants

Thirty five confirmed positive female cases willing to be the part of the study were enrolled. Positive confirmatory cases of COVID-19 infection was defined as those with a positive

RT-PCR assay of nasal/oropharyngeal test result from Institute's laboratory or from any ICMR (Indian Council of Medical Research) accredited laboratory.

Procedure

Initially 35 confirmed cases were considered as a part of interim analysis, which was to be further increased provided positive result was obtained. This study was reviewed and approved by the institute's ethics committee (IEC/04/466/29.05.2020). Written informed consent was obtained from each enrolled patient. Partners' COVID-19 status was enquired. The gynaecological and obstetrical history, clinical characteristics, laboratory findings of the patients were recorded in detail. Special emphasis was given for sexual history especially regarding sexual intercourse in the past 14 days. Vaginal discharge samples from patients with COVID-19 were obtained from posterior fornix of vagina according to the protocol of the virus sampling kit. All the patients from whom the vaginal samples were collected were admitted within a day or two of their initial onset of symptoms and their vaginal sample sent within 24 h of admission, implying the samples were collected during the acute phase of infection. The flocced nylon swabs were inserted 2–3 cm into the vagina and rotated for 3–5 s. The samples collected were placed in vials containing 2 mL of viral transport media and immediately transported to virology laboratory of the institute along with icepacks to keep it at around (2°C–8°C) as per standard protocol. The viral transport medium consists of Hanks Balanced Salt Solution modified and enriched with bovine serum albumin, amino acid, gelatin peptone and carbohydrate. The pH (7.3 ± 0.2) is adjusted with buffer. Phenol red is used as a pH indicator. Vancomycin, amphotericin B and colistin have been added to the medium to inhibit the proliferation of competing bacteria and yeasts. The medium is isotonic and lacks toxicity to the mammalian host cells.

All samples were subjected to RNA extraction by silica membrane-based spin column technology using Qiagen viral RNA Minikit, (Qiagen catalogue no. 74104, USA). About 300 µL of whole samples was used for RNA extraction according to kit protocol. The RNA samples were kept at –20°C for further analysis.

RT-PCR

RNAs extracted from all the samples were run in real-time PCR machine (Biorad CFX 96) using approved one-step real-time RT-PCR kits approved by ICMR, Govt of India.

The RT-PCR was performed according to kit guidelines, for that primer and probe mix kit was used which adopts the dual-target gene design, which targets the specific conserved sequences encoding the ORF 1 laboratory gene and the nucleoprotein N gene with the PCR reaction mix provided, the amplification of template is quantitatively monitored by the

Table 1. Demographic profile of the patients.

Age, years	<30	n= 18 (51.42%)
	30–40	n=9 (25.71%)
	40–50	n=5 (14.28%)
	>50	n=3 (8.57%)
BMI (kg/m ²)	<20	n= 15 (42.85%)
	20–24	n= 10 (28.57%)
	25–29	n=5 (14.28%)
	>30	n=5 (14.28%)
Menstrual history	Pre-menarche	n=0 (0%)
	Menstruating	n=27 (77.14%)
	Post-menopausal	n=5 (14.28%)
	Pregnancy	n=2 (5.71%)
	Post-partum	n= 1 (2.85%)

BMI: body mass index.

increasing fluorescence signal detected by real-time thermo cycler (Biorad CFX 96). All the runs were validated by appropriate positive control and no template control. All the samples were processed in Biosafety label 2 laboratory facilities.

Statistical methods

Descriptive statistics were used as appropriate. The continuous variables were presented as mean and categorical variables as count (%).

Patient and public involvement

Neither patients nor the public were involved in the design and conduct of our research. Patients were only involved as participants in the research.

The results of the study however will be sent to the institute's website to the related patients and to the funders. A lay summary will be created and published at the institute's website.

Results

Thirty five COVID-19-positive female patients were recruited in this study.

The range of their age was 21 to 70 years, with a mean age of 33.08 years. Most of the patients had body mass index (BMI) <20. No pre-menarche patients were included in our study.

The number of menstruating women taken in the study was n=27 (77.14%), with number of patients who had physiological menopause n=5 (14.28 %). Two vaginal swab samples were obtained from two pregnant women prior to caesarean section (n=2, 5.71%).

One vaginal swab was obtained post-partum (3 days after caesarean section) (n=1, 2.85%) (Table 1).

Out of 35 patients, the number of sexually active patients was n=30 (85.71%). All the 35 patients were not hesitant to reveal their sexual history. Eighteen female patients admitted

having sex with their partners during a possible infection incubation period (n=18, 51.42%), while the number of patients who did not have sex in the past 14 days was n=12 (34.28%). The number of patients whose partner was positive was n=9 (25.71%), while the number of patients whose partner was negative was n=24 (68.57%). Out of the nine patients whose partner was positive, the index case-partner was (n=4, 44.44%), and the index case-patient was (n=5, 55.55%). Spouse of two patients were already deceased (Table 2).

Vaginal secretions from the 35 female patients which were tested for COVID-19 were all negative for SARS-CoV-2 (Table 3).

Discussion

In our study, of 35 patients, with COVID-19-positive status, all vaginal fluids with RT-PCR assay tested negative for the virus. Previous studies have explored the effects of other epidemic viral infections on the female reproductive tract. Murray et al.⁷ confirmed the presence of Zika virus in the female reproductive tract. Rodriguez et al. found Ebola virus in the vaginal fluid of a patient recovering from Ebola virus infection 33 days after the onset of illness.^{8,9} Unlike Ebola and Zika virus, SARS-CoV-2 is not found in the vaginal fluids of women. Therefore, it is possible that the virus does not enter the vaginal fluid. This finding suggests that the likelihood of transmitting SARS-CoV-2 to sexual partners through the vaginal fluids may be low. Also, since no SARS-CoV-2 virus was found by RT-PCR in the vaginal fluids; therefore, it is speculated that the risk of vertical transmission during vaginal delivery might also be very low. To the best of our knowledge, no such study was conducted in India and South East Asian Region (SEAR). On reviewing the literature, we found a study from Wuhan, China, where researchers had investigated for presence of virus in cervical secretions and anal swab in 33 patients. They found all 33 cervical and vaginal secretions to be negative for the virus, and only one anal swab tested positive for SARS-CoV-2. Qiu et al.¹⁰ reported that 10 women in intensive care unit (ICU) with severe COVID-19 were tested for SARS-CoV-2 virus in the vaginal fluid by RT-PCR, and all the samples were negative for the virus. Also Aslan et al.¹¹ reported that among 12 confirmed COVID-19-positive females, none of the vaginal fluids tested positive for the virus. The possible reason could be because SARS-CoV-2 infection is dependent on both angiotensin-converting enzyme 2 (*ACE2*) and transmembrane serine protease 2 (*TMPRSS2*) expression for the entry of the virus into the host cell, both of which are expressed less in female genital tract.

In our study, although it cannot be definitely concluded, but the possibility of the female patient being the index case and transmitting the virus to her male partner via vaginal sex is unlikely since no virus has been isolated from the vaginal secretions. However, there is every possibility of the male partner being the index case and transmitting the virus to her female partner via vaginal sex since there are evidences of

Table 2. Sexual behaviour and partner status.

Sexually not active			n = 5 (14.28%)
Sexually active			n = 30 (85.71%)
Patient not willing to disclose sexual behaviour			n = 0 (0%)
Patient had intercourse within past 14 days			n = 18 (51.42%)
Patient who did not have sexual intercourse in past 14 days			n = 12 (34.28%)
Partner status	Positive (n = 9, 25.71%)	Index case-partner	n = 4 (44.44%)
	Negative	Index case-patient	n = 5 (55.55%)
	Partner deceased		n = 2 (5.71%)

Table 3. SARS-Cov-2 test by RT-PCR of throat swab and exfoliated cells from cervix or vagina, Sexual behaviour of the patients, Partners status of the patients.

Patient no.	Throat swab (RT-PCR)	Exfoliated cells from cervix or vagina (RT-PCR)	Sex behaviour	Partner status
1	Positive	Negative	Sexually active(+)	Negative
2	Positive	Negative	Sexually active(+)	Negative
3	Positive	Negative	Sexually not active	Deceased
4	Positive	Negative	Sexually active(-)	Negative
5	Positive	negative	Sexually active(+)	Negative
6	Positive	Negative	Sexually active(+)	Negative
7	Positive	Negative	Sexually active(-)	Positive
8	Positive	Negative	Sexually active(+)	Negative
9	Positive	Negative	Sexually active(-)	Negative
10	Positive	Negative	Sexually active(+)	Negative
11	Positive	Negative	Sexually not active	Positive
12	Positive	Negative	Sexually active(+)	Positive
13	Positive	Negative	Sexually active(+)	Negative
14	Positive	Negative	Sexually active(+)	Positive
15	Positive	Negative	Sexually active(+)	Negative
16	Positive	Negative	Sexually not active	Positive
17	Positive	Negative	Sexually active(-)	Negative
18	Positive	Negative	Sexually active(+)	Negative
19	Positive	Negative	Sexually active(-)	Positive
20	Positive	Negative	Sexually active(-)	Negative
21	Positive	Negative	Sexually not active	Deceased
22	Positive	Negative	Sexually active(-)	Negative
23	Positive	Negative	Sexually active(-)	Positive
24	Positive	Negative	Sexually active(-)	Negative
25	Positive	Negative	Sexually active(+)	Positive
26	Positive	Negative	Sexually active(+)	Negative
27	Positive	Negative	Sexually active(+)	Negative
28	Positive	Negative	Sexually not active	Negative
29	Positive	Negative	Sexually active(+)	Negative
30	Positive	Negative	Sexually active(+)	Negative
31	Positive	Negative	Sexually active(-)	Negative
32	Positive	Negative	Sexually active(-)	Negative
33	Positive	Negative	Sexually active(-)	Negative
34	Positive	Negative	Sexually active(+)	Positive
35	Positive	Negative	Sexually active(+)	Negative

RT-PCR: reverse transcriptase polymerase chain reaction; +: stands for had sex recently; -: stands for did not have sex recently.

the presence of virus in the semen. Li et al. (2020) have reported the presence of SARS-CoV-2 in the semen samples collected from six patients (out of 38 patients) with COVID-19, including two recovering individuals.¹²

Our study did not evaluate the semen of the male partner especially in those where male partner were the index case (n=4). Hence, we limit the conclusion of our study upto the presence or absence of virus in the cervico-vaginal secretions

of COVID-19-affected female and not comment much on the possibility of sexual transmission.

Sexual behaviour in a more broad perspective involves other intimate contacts involving hugs, kisses, and oral/anal sex. The possibility of the virus being passed on to the partner by these intimate contacts should also be kept in mind. In this study, no evidence indicated that SARS-CoV-2 could transmit by vaginal sex from female to her partner. But the risk of infection by oral/anal sex or other intimate contacts during sex should not be ignored and hence it is best to avoid during the infectivity period.

Despite the prolonged nature of the pandemic and the number of people infected worldwide, there still are limited data on the effects of the virus and infection on human reproductive health and further research is needed.^{13,14}

Limitations

Samples from male partner of the patients enrolled in the study are missing, including anal swabs, semen and urethral orifice swabs. These samples were important especially in those where the partner were the index case (n=4).

In addition, the sensitivity and specificity of the RT-PCR test is not full proof which may lead to non-detection of some RNA particles in the vaginal swab. Correlation between the cycle threshold (Ct) value –calculated viral load and viral shedding in the body fluids and hence its infectivity has not been done in our study which could be a critical factor.

The sample size of the study is small. The power of analysis for sample size calculation was not done. An initial sample of 35 patients was taken for interim analysis, which was to be further increased if any significant result was obtained.

Drawing conclusion from a small sample size may not be apt.

Conclusion

Findings from this study suggest that SARS-CoV-2 virus does not exist in the vaginal fluids of Covid-19 patients since no positive RT-PCR result was found in the vaginal environment. The results from this study show transmission of SARS-CoV-2 through vaginal sex from female to her male partner is less likely. However, the risk of infection of non-vaginal sex and other intimate contacts during vaginal sex should not be ignored. Also, there is every possibility of the male partner being the index case and transmitting the virus to her female partner via vaginal sex since there are evidences of the presence of virus in the semen. Keeping these facts in mind, it is best to avoid sexual activities during the infectivity period until more robust studies are available.

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Author contributions

M.A. conceptualised the study, wrote the manuscript, did the initial drafting, analysed the data and finalised the drafting. S.B. helped in writing the manuscript, was involved in collecting the samples and helped in finalising the draft. D.B. clinically managed the management and helped in the finalising the draft. B.K.P. contributed in processing and furnishing the report of the samples.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

Ethical approval for this study was obtained from Institutional Ethical Committee—AIIMS, Patna (IEC/04/466/29.05.2020).

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Informed consent

Written informed consent was obtained from all subjects before the study.

ORCID iD

Mukta Agarwal  <https://orcid.org/0000-0002-0141-5756>

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