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# The presence of SARS-CoV-2 virus in semen samples of patients with COVID-19 pneumonia

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## Abstract

The SARS-CoV-2 set off a pandemic involving millions of people around the world. The topic of discussion is the possible viral detection in different body fluids than respiratory droplets. Therefore, we evaluated the possible presence of SARS-CoV-2 in semen and urine samples. Thirty patients were included in the study aged 35.67 ± 6.84 years. The day after the pharyngeal and/or nose swab of SARS-CoV-2 was positive, urine and semen samples were taken from patients, and the presence of SARS-CoV-2 was investigated. Laboratory tests and chest CT findings were evaluated simultaneously. SARS-CoV-2 was detected in four (13.3%) patients' semen samples and in seven (23.3%) patients' urine samples. White blood cell (WBC), neutrophil, C-reactive protein (CRP), ferritin, alanine transaminase (ALT), lactate dehydrogenase (LDH) and procalcitonin were significantly higher in patients with SARS-CoV-2 in semen ($p < .05$), though no statistical difference was found in urine ($p > .05$). Patients with severe pneumonia findings in Chest CT images are likely to be PCR positive in semen and urine samples ($p = .005$, $p = .001$). SARS-CoV-2 was not detected in urine and semen samples of patients after they had recovered (average duration 23 ± 4 days). SARS-CoV-2 can be detected in the urogenital fluids of patients with severe clinical conditions and high viral load.

**KEYWORDS**
COVID-19, SARS-CoV-2, semen, urine

## 1 | INTRODUCTION

Coronavirus (SARS-CoV-2) induced a pandemic all over the world, causes severe respiratory failure with high mortality rates. It also causes a systemic disease (COVID-19) that affects the cardiovascular, gastrointestinal and urogenital systems (Çayan et al., 2020). In men, the disease is longer lasting and clinically more severe. Besides, the incidence and morbidity of the disease are two times higher in men (Huang et al., 2020). Researchers have done many studies on viral transmission pathways. The transmission routes of this disease, which affects the entire world, have gained great importance. SARS-CoV-2 can be transmitted directly by coughing, sneezing and droplet inhalation, or through contact with ocular contact, saliva, mucous membranes of the nose and eyes (Baghizadeh Fini, 2020). SARS-CoV-2 uses ACE2 (angiotensin-converting enzyme 2) as an important receptor for entry into target cells. The transmembrane protease serine 2 (TMPRSS2), which mainly acts as a degrading enzyme, helps the spike protein of the SARS-CoV-2 virus to attach to the ACE2 receptors and allows the fusion of the virus and the host cell membrane by breaking down the viral S protein. TMPRSS2 cleaved the ACE2 receptor and allows the virus to enter the cell. In other words, for the virus to infect the cell, not only the ACE2 receptor is sufficient, but also the TMPRSS2 (Achua et al., 2021). A virus entering the cell leaves

  

its virions out of the cell by exocytosis. The amount of ACE2 receptors and TMPRRS 2 expressions of the cells determines which tissues will be involved in this viraemia stage. For this reason, the lung, myocardium, proximal tubule and ileum epithelium are mostly affected and a disease of the system occurs due to the dysfunction of the involved organs. In semen, no clear conclusion was reached in the literature. Although the small sample size in the studies sheds light on the literature, a broad range of viral families found in human spermatozoa suggests that the SARS-CoV-2 virus may be present in human semen and seminal fluid (Salam & Horby, 2017). ACE2 receptors are abundant in the testicles (Huang et al., 2020). The only known transcription promoters for the TMPRSS2 gene are androgens; they do this through androgen receptors (Goren et al., 2020; Wambier & Goren, 2020). Both the high expression of ACE2 and TMPRSS2 in testicular tissue and high androgen presence in testicular tissue may allow the viral entry of SARS-CoV-2 (Barbry et al., 2020). Therefore, we thought that there may be a SARS-CoV-2 virus in semen. We investigated the presence of the virus in laboratory-confirmed SARS-CoV-2 patients' semen.

## 2 | MATERIALS AND METHODS

This study was performed between 10 and 25 November 2020 in Mersin City Training and Research Hospital. All patients who participated in the study were informed about the study and gave written consent. Approval was obtained from the Ministry of Health and then from Mersin University Ethics Committee for the study (2020/461). Thirty patients aged between 18 and 50 years with laboratory-confirmed SARS-CoV-2 were included in the study. Those with urogenital diseases affecting the testes such as cryptorchidism, atrophic testis, varicocele and hypogonadism were not included in the study. Blood tests and chest CT scans were taken routinely from all patients.

The day after the PCR test was positive, urine and semen samples were taken from patients during the viraemia period of the disease. Semen samples were obtained by masturbation considering the patient's physiological and psychological condition. All patients put on their masks and washed their hands thoroughly before giving samples and gave semen and urine samples at separate times to prevent possible contamination. Urine and semen samples were taken by adding the same amount (0.2 ml) to 0.2 ml Bio-Speedy VNAT Transfer tube with Lot number 2B01024SD. The samples were transferred at +2-8°C until they reached the laboratory, frozen and stored at −20°C until the study was carried out. PCR work was performed with the Bio-Speedy SARS-CoV-2 double Gene RT-qPCR kit numbered 2b01124ce100r250 lot. Diagnosis with Kit was performed by single-step reverse transcription (RT) and real-time PCR (qPCR) (RT-qPCR) targeting the SARS-CoV-2 specific N and orf1ab gene region. The results of the study were studied on the Bio-Rad CFX96 Touch device and evaluated by the kit's user manual. The sensitivity of this method was 99.4%, and the specificity was 99%.

On chest computer tomographic (CT) scan (GE Healthcare Optima CT660, Chicago, IL, USA), positive radiological findings of the patients were evaluated by the same radiologist. Depending on the lung findings of SARS-CoV-2, patients were divided into three groups by using the British Society of Thoracic Imaging (BSTI) guideline statement category as, Non-COVID-19 (group 1), indeterminate and probable COVID-19 (group 2) and classic COVID-19 (group 3) (Inui et el., 2020). In patients with SARS-CoV-2 in the semen samples, PCR was performed in the semen samples again before discharge (average time 3 weeks). On the day they gave a semen sample, the same laboratory tests were studied from the patients.

For statistical analyses, SPSS (Statistical Package for the Social Sciences Inc, Chicago, IL, USA) version 21.0 package program was used, and $p$ values less than 0.05 were considered to be statistically significant. Descriptive statistics for continuous variables were expressed and also tabulated as mean ± standard deviation, and for categorical variables as frequencies, and percentages (%). Mann–Whitney $U$ non-parametric test and paired samples test were used to compare laboratory tests.

## 3 | RESULTS

The average age of the 30 patients who participated in the study was 35.67 ± 6.84 years. The patients were aged between 23 and 47 years. SARS-CoV-2 was detected in four (13.3%) patients' semen samples and in seven (23.3%) patients' urine samples. The age of the patients did not have a statistically significant effect on the detection of SARS-CoV-2 in semen and urine samples (Mann–Whitney U non-parametric test, $p = .079$, $p = .737$). Six of the patients included in the study had a fever. There was no fever in patients with SARS-CoV-2 in the semen sample. White blood cell (WBC), neutrophil, C-reactive protein (CRP), ferritin, alanine transaminase (ALT), lactate dehydrogenase (LDH) and procalcitonin were significantly higher in patients with SARS-CoV-2 in semen ($p < .05$) (Table 1). No statistically significant change was found between the detection of SARS-CoV-2 in the urine sample of patients and laboratory parameters ($p > .05$). Laboratory parameters on the day of taking the sample of PCR-positive patients in the semen sample and laboratory parameters on the day of negative results in the semen sample before discharge were compared by using paired samples test. A statistically significant increase was found in WBC, neutrophil, CRP, procalcitonin, ferritin, ALT and LDH values on the day when PCR was positive in the semen sample ($p < .05$). No statistically significant difference was found between laboratory parameters on the days when patients were PCR positive and negative in urine samples ($p > .05$). It was found that the SARS-CoV-2 detection rate was statistically increased in urine and semen samples in those with BSTI classic COVID-19 category ($p = .005$, $p = .001$) (Table 2). PCR performed again before discharge after PCR-positive patients recovered (average duration 23 ± 4 days). SARS-CoV-2 was not detected in the urine and semen samples of the patients after they recovered.

**TABLE 1** Laboratory parameters of SARS-CoV-2 PCR-positive and PCR-negative patients in the semen sample. (Mann–Whitney U non-parametric test used to compare laboratory values between SARS-CoV-2 PCR-positive and PCR-negative patients in the semen sample)

| | Semen PCR | *n* | Mean | *SD* | SE | *p*-Value |
|---|---|---|---|---|---|---|
| WBC | Positive | 4 | 13,397.50 | 1,495.78 | 747.89 | .000 |
| | Negative | 26 | 6,532.69 | 1785.32 | 350.13 | |
| Neutrophil ( /L) | Positive | 4 | 6,442.50 | 1,403.29 | 701.64 | .001 |
| | Negative | 26 | 3,571.85 | 1,197.69 | 234.88 | |
| CRP (mg/L) | Positive | 4 | 3.98 | 0.68 | 0.34 | .026 |
| | Negative | 26 | 1.95 | 1.66 | 0.32 | |
| Ferritin (mg/L) | Positive | 4 | 797.75 | 119.58 | 59.79 | .004 |
| | Negative | 26 | 534.62 | 218.88 | 42.92 | |
| D-dimer (mg/L) | Positive | 4 | 0.71 | 0.45 | 0.22 | .930 |
| | Negative | 26 | 0.74 | 0.36 | 0.07 | |
| ALT (U/L) | Positive | 4 | 79.50 | 12.89 | 6.44 | .018 |
| | Negative | 26 | 57.31 | 17.93 | 3.51 | |
| Creatinine (mmol/ml) | Positive | 4 | 0.90 | 0.16 | 0.08 | .359 |
| | Negative | 26 | 0.86 | 0.14 | 0.02 | |
| LDH (mU/L) | Positive | 4 | 903.75 | 55.58 | 27.79 | .001 |
| | Negative | 26 | 606.04 | 213.57 | 41.88 | |
| Procalcitonin (mg/L) | Positive | 4 | 0.27 | 0.05 | 0.02 | .011 |
| | Negative | 26 | 0.16 | 0.06 | 0.01 | |

Abbreviations: ALT, Alanine aminotransferase; CRP, C-reactive protein; LDH, Lactate dehydrogenase; *SD*, Standard deviation; SE, Standard error mean; WBC, White blood cell.

**TABLE 2** Chest computerised tomography findings of patients with SARS-CoV-2 in semen and urine samples

| **Patients with SARS-CoV-2 in urine samples** | | | |
|---|---|---|---|
| | **Urine sample PCR test** | | |
| | **Negative *n* (%)** | **Positive *n* (%)** | ***p*-Value** |
| Chest CT findings | | | |
| Group 1 | 5 (100) | 0 (0.0) | .001 |
| Group 2 | 15 (93.8) | 1 (6.2) | |
| Group 3 | 3 (33.3) | 6 (66.7) | |
| **Patients with SARS-CoV-2 in semen samples** | | | |
| | **Semen sample PCR test** | | |
| | **Negative *n* (%)** | **Positive *n* (%)** | ***p*-Value** |
| Chest CT findings | | | |
| Group 1 | 5 (100) | 0 (0.0) | .005 |
| Group 2 | 16 (100) | 0 (0.0) | |
| Group 3 | 5 (55.6) | 4 (44.4) | |

Abbreviation: CT, Computerised tomography.

## 4 | DISCUSSION

It has been reported that the main transmission routes of the SARS-CoV-2 virus are the droplet pathway and close contact but maybe in a small amount of faecal-oral transmission (Li, Guan, et al., 2020). There are many studies in the literature on possible transmission routes of the SARS-CoV-2 virus. No clear conclusions have been reached, especially regarding the detection of the SARS-CoV-2 virus in semen. In many studies in the literature, no virus was detected in semen samples of SARS-COV-2 patients (Kayaaslan et al., 2020; Pan et al., 2020; Paoli et al., 2020; Song et al., 2020). In contrast to these findings, in a study conducted by Li and colleagues in March 2020, they investigated the presence of the virus in semen in a total of 38 patients, 15 of whom were in the acute stage, and obtained positive

results in six patients (Li et al., 2020). However, in their study, the method of taking semen samples and how they were analysed was not clearly stated. The presence of the virus in the urine of the same patients was not investigated, and possible urinary semen transmission could not be ruled out (Holtmann et al., 2020; Paoli et al., 2021). In our study, semen and urine samples were taken from the patients simultaneously and all patients put on their masks and washed their hands thoroughly before giving samples. In this way, possible urine semen contamination was prevented. To prevent viral RNA contamination that may occur with cough, patients wore masks during masturbation. However, contamination cannot be prevented absolutely and it can play a role in the final results.

The presence of SARS-CoV-2 in semen has been investigated in a limited number of studies, and mostly in recovering patients. Patients who received semen samples in literature studies either had a mild clinic or were discharged from the hospital (Punjani et al., 2020). Disease symptoms may be more severe in patients with a higher viral load (Liu et al., 2020). Viruses that can cause viraemia can cross the blood–testicular barrier in the presence of systemic or local inflammation (Perry et al., 2021).

As a result of the uncontrolled release of cytokines, the situation we describe as cytokine storm occurs. Especially pro-inflammatory cytokines (IL-1, IL-6, IL-10, IFN-γ, TNF-α) are particularly effective in a cytokine storm. Cytokine storm occurs as a clinical result of systemic inflammation, multiple organ failure, hyperferritinaemia and death (Soy et al., 2020). When the laboratory tests showing the course of cytokine storm in the follow-up of the patients were examined, it was found that ferritin levels remained below 1,000 μg/L, no cytopenia developed; CRP values remained under control, and no progression was observed. None of our patients had acute respiratory distress syndrome (Henderson et al., 2020). We could not have an idea about IL-6 levels, because IL-6 examination could not be studied within the hospital's facilities. However, in clinical and laboratory follow-up, cytokine storms did not develop in our patients. All patients were followed up in the COVID services and were not taken into intensive care units.

So, does the SARS-CoV-2 virus cross the blood–testicular barrier? In a recent study, researchers have shown that the SARS-CoV-2 virus in the testicles in autopsy biopsies of patients who died was due to the COVID-19 (Achua et al., 2021). In the same way, we think that the virus can cross the blood–testicular barrier. Additionally, the margin of false negativity rate in test results is 15%–20% and these results may be due to errors in sampling and differences in the PCR test methods applied (Walsh et al., 2020).

In our study, SARS-CoV-2 was detected in the semen of four of 30 patients. Patients with SARS-CoV-2 in semen were in the acute stage of the disease and have severe pneumonia. CT scans of the patients showed severe pneumonia and a typical ground-glass opacity (BSTI classic COVID-19 category). Also, laboratory values were worse than the other patients. According to clinical findings, the virus load of these patients was much higher than other patients. These four patients were then taken to the intensive care unit. SARS-CoV-2 was not detected in the urine samples of three of the four patients who received positive results in their semen samples. In other words, there was no risk of urine contamination in three of the four patients with a virus detected in semen samples. Then, after these patients recovered, semen samples were taken before discharge. The SARS-CoV-2 virus was not detected in the semen of the same patients. The average time of taking the second sample was 23 ± 4 days, but we cannot say anything clear about how long the virus stays in the semen.

The results found in our study show that SARS-CoV-2 may be in the semen samples of patients with severe pneumonia. However, it was not found in the semen samples of patients with the milder clinical disease who did not need intensive care with a less viral load. The SARS-CoV-2 virus in semen may come from the seminal fluid or spermatozoa in the testicles, which something we cannot say clearly in our current study. ACE2 receptor and TMPRSS2 level in the seminal vesicle are low, so the possibility of the SARS-CoV-2 virus entering the seminal vesicle is low (Massarotti et al., 2021). We think it is from the testicles for these two reasons.

## 5 | CONCLUSION

The most important feature of our study that distinguishes it from other studies is that samples can be taken from patients with more severe clinical disease and urine samples were taken simultaneously. SARS-CoV-2 can be detected in semen samples of patients with severe clinical conditions and high viral load. We have shown the presence of the SARS-CoV-2 virus in semen, but we still do not know whether it can be sexually transmitted or not. However, at the severe pneumonia stage, the patients can transmit the virus through saliva and droplets. We also do not know how active replication capacity of the SARS-CoV-2 virus in semen is and whether it can infect the cells it reaches or not. Further studies need to be done to show whether SARS-CoV-2 can be transmitted with isolated sexual intercourse. In the future, as the diagnostic methods improve and the number of studies increases, we will have more information about the presence of the SARS-CoV-2 virus in urogenital fluids.

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None.

### CONFLICT OF INTEREST

The authors declare no conflict of interest regarding this study.

### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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