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Short Communication

The absence of coronavirus in expressed prostatic secretion in COVID-19 patients in Wuhan city

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ABSTRACT

Due to the cellular entry of the novel coronavirus (SARS-CoV-2) modulated by angiotensin converting enzyme 2 (ACE2), the ACE2 bearing prostate is therefore hypothesized as a susceptible organ to COVID-19. To delineate whether the pathogenic SARS-CoV-2 of the coronavirus disease (COVID-19) could be detected in the expressed prostatic secretion (EPS), a total of ten male patients with confirmed COVID-19 were recruited. All patients were stratified into two groups: one group with positive nasopharyngeal swabbing SARS-CoV-2 within 3 days of the EPS taken day (PNS group, n = 3) and the other group with previously positive nasopharyngeal swabbing SARS-CoV-2 but turned negative before the taken day (PNNS group, n = 7). The COVID-19 patients showed elevated inflammatory indictors, i.e. C-reaction protein (3.28 (1.14, 33.33) mg/L), erythrocyte sedimentation rate (22.50 (8.00, 78.50) mm/h), and interleukin-6 (6.49 (4.96, 21.09) pg/ml). Serum IgM against SARS-CoV-2 was only positive in the PNS group, whereas serum IgG was positive for all patients. Furthermore, our data showed for the first time that none of the COVID-19 patients had positive SARS-CoV-2 RNA in EPS. To this end, this study found the negativity of SARS-CoV-2 in EPS and possibly exclude the sexual transmission of COVID-19.

1. Introduction

Currently, the novel coronavirus disease (COVID-19) has outbroken in China and exponentially spread along the world since the first case was diagnosed on December 2019 in Wuhan City of China [1]. The pathogenic novel coronavirus (SARS-CoV-2), isolated from the nasal and pharyngeal secretion, was highly homologous with the coronavirus caused Severe Acute Respiratory Syndrome (SARS) [2]. Owing to the lack of immunity this global pandemic increases sharply within a few months, which not only burns a large amount of health expenditure but seriously threatens lives.

The amputating of COVID-19 transmission route is one of the effective measures to prevent the continuing spread. This disease is believed to transmit by inhalation or contact with infected droplets, which is confirmed by the detection of coronavirus in nasopharyngeal swabs [3] and saliva [4] of COVID-19 patients. Apart from the respiratory tract, the SARS-CoV-2 could be also detected in urine and gastrointestinal tract (positive percentage: urine: 0.076%; stool: 0.30%; rectal swab: 0.30%) [5]. Yet, to the best of our knowledge the detection of SARS-CoV-2 in the prostate or expressed prostatic secretion (EPS) has not been reported up till now.

Angiotensin converting enzyme 2 (ACE2) was firstly found as a modulator involved in the regulation of renin-angiotensin aldosterone system (RAAS) [6]. It distributes specifically in alveolar epithelial cells, enterocytes of the small intestine and testis in SARS pathogenesis [7]. ACE2 bearing cells are found to be more vulnerable to the attack of coronavirus since the coronavirus spike protein mediates the viral entry to its target cells [8,9]. Indeed, ACE2 has been proved to be a putative

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Table 1
The demographic and clinical data of ten male patients.

		Normal range
N	10	NA
Age (yeas)	57.50 (38.75, 69.25)	NA
Height (cm)	170.00 (167.50, 172.00)	NA
Body weight (kg)	70.00 (61.50, 80.50)	NA
BMI (kg/m^2)	24.81 (21.28, 27.58)	18.5 - 23.9
Maximum Temperature (℃)	39.00 (38.60, 39.23)	36 - 37
Pulse (beats per minute)	96.00 (84.25, 115.50)	60 - 100
Respiration (times per minute)	20.00 (18.75, 22.00)	12 - 20
Systolic blood pressure (mmHg)	127.50 (115.75, 139.25)	90 - 140
Diastolic blood pressure (mmHg)	90.50 (69.25, 96.00)	60 - 90
Blood oxygen saturation (%)	96.50 (93.50, 99.00)	> 90%
The interval between the emerge of symptoms and diagnosis (days)	11.00 (8.25, 17.00)	NA
Tumor history (n)	0	NA
Heart failure history (n)	0	NA
Cerebrovascular disease history (n)	0	NA
Kidney disease history (n)	0	NA
Liver disease history (n)	2 (20.00%)	NA
Diabetes (n)	5 (50.00%)	NA
Hypertension (n)	2 (20.00%)	NA
Chronic obstructive pulmonary disease	1 (10.00%)	NA
Smoking history (n)	6 (60.00%)	NA
Alcohol intaking history (n)	8 (80.00%)	NA
Nursing house staying history (n)	0	NA
Unconsciousness (n)	0	NA
Pleural effusion (n)	1 (10.00%)	NA

Abbreviations: BMI: body mass index; NA: not applicable.

receptor for COVID-19 recently [8]. This probably explains the pathogenesis of lung and intestine during SARS and COVID-19. The prostate is also one of the organs express abundant ACE2 [10]. Therefore, it is reasonable to hypothesize that the prostate may be affected by SARS-CoV-2. In this case male fertility could be influenced, and sexual transmission also needs to be taken into account. Yet, recent studies have found the absence of SARS-CoV-2 in the semen and testis [11,12] although the testis is an ACE rich organ. Hence, we aimed to delineate in this study whether SARS-CoV-2 could be detected in EPS and if so, whether COVID-19 was transmitted via sex behavior. Our study was also designed to further prove the initial finding concerning the absence of the virus in semen as the EPS is an essential part of it. The clinical features of these patients were also analyzed, with the focus on the inflammatory indicators and serum SARS-CoV-2 immunoglobins.

2. Materials and methods

2.1. Subjects

A total of 10 male inpatients with confirmed diagnosis of NCP were recruited from quarantine wards in the Cancer Center, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430022, China.

The diagnosis of COVID-19 was confirmed by either 1) the real-time reverse-transcriptase polymerase-chain-reaction (RT-PCR) or sequencing for nasopharyngeal swabbing SARS-CoV-2 RNA [13], or 2) the positive serum immunoglobin M (IgM) or IgG antibodies of coronavirus. Patients with NCP was the patients confirmed with both COVID-19 and pneumonia diagnosed by CT scan.

Patients over 80 years or concomitant with prostatic cancer were excluded.

The maximum temperature was recorded according to the medical history given by each patient, but the pulse, respiration, blood pressure and other routine inspections were recorded at admission. The serological parameters were basically collected the second day after admission.

All subjects gave their oral and written informed consent before recruitment. This project is approved by the local ethical committee (No. Quick-PJ2020-03-35) in terms of urgent need.

2.2. The two-glass test for EPS

Although the Meares-Stamey 4-glass test is the standard method to obtain EPS, there was a study showed that the pre- and post-massage test has strong concordance with the 4-glass test [14]. Considering the time and the cost, the EPS in our study was obtained pre- and post-palpation of the prostate by digital rectal examination, which is also called “two-glass test”. Abstinence was required for at least 5 days before examination. The two operating doctors had been well trained by the urologists beforehand. The detailed operation included two steps. The initial step was the wearing of protective clothing, including the goggle and protective facial screen, one KN95 facial mask plus one surgical facial mask, three surgical gloves, three shoe covers, one protective suit and one isolation gown. All procedures were supervised by the trained staffs from the Centers for Disease Control (CDC). The second step for the two-glass test was the digital rectal examination. The patients were asked to take a knee chest position after the disinfection of external urethral orifice. The urine was collected hereafter as “the first glass of urine”. Afterwards, the doctors inserted the index that lubricated with paraffin oil to the recta and palpated the prostate gently. The EPS was collected as “the second glass of EPS”.

The RT-PCR kit (DAAN Gene Co, Ltd of Sun Yat-sen University) was used for SARS-CoV-2 RNA detection in EPS. To ensure the consistent positive nasopharyngeal SARS-CoV-2, nasopharyngeal swabbing was retested within 3 days of the EPS taken day. Serum IgM and IgG were also tested.

A questionnaire on sociodemographic and sexual behaviors would be conducted if the EPS was positive.

2.3. Statistical analysis

Quantitative data were depicted as median and interquartile range (Percentile 25, Percentile 75). Statistical analyses were performed with SPSS26.0.

Table 2
The serological data of ten male patients.

		Normal range
White blood cells ($\times 10^9$/L)	4.94 (4.06, 5.47)	3.50 - 9.50
Neutrophil ($\times 10^9$/L)	2.97 (2.28, 3.52)	1.80 - 6.30
Lymph cells ($\times 10^9$/L)	1.43 (0.57, 1.79)	1.10 - 3.20
Hematocrit (%)	36.90 (33.88, 40.85)	40 - 50
Platelet ($\times 10^9$/L)	204.50 (178.75, 272.25)	125.00 - 350.00
Hydrogen carbonate (mmol/L)	23.95 (22.18, 26.23)	21.00 - 30.00
Sodium (mmol/L)	139.00 (137.39, 139.81)	136.00 - 145.00
Urea nitrogen (mmol/L)	4.75 (4.09, 5.45)	2.90 - 8.20
Creatinine (μmol/L)	88.00 (69.75, 92.75)	44.00 – 133.00
eGFR (ml/min)	88.01 (78.54, 116.25)	> 90
Fasting blood glucose (mmol/L)	5.41 (4.95, 8.01)	3.90 - 6.10
HbA1c (%) *	8.80 (6.70, 9.35)	4.50 - 6.20
Total bilirubin (μmol/L)	12.75 (8.23, 20.73)	5.10 – 19.00
Alanine aminotransferase (u/L)	36.50 (26.25, 62.50)	5.00 – 40.00
Total cholesterol (mmol/L)	4.34 (3.71, 4.82)	< 5.20
Triglyceride (mmol/L)	1.15 (0.98, 1.62)	< 1.70
Troponin (ng/L)	2.25 (1.55, 5.63)	< 26.20
C-reaction protein (mg/L)	3.28 (1.14, 33.33)	< 4.00
Procalcitonin (ng/ml)	0.075 (0.065, 0.105)	< 0.50
ESR (mm/h)	22.50 (8.00, 78.50)	0.00 - 15.00
Interleukin 2 (pg/ml)	3.94 (2.10, 4.31)	0.10 - 4.10
Interleukin 4 (pg/ml)	2.73 (1.73, 4.08)	0.10 - 3.20
Interleukin 6 (pg/ml)	6.49 (4.96, 21.09)	0.10 - 2.90
Interleukin 10 (pg/ml)	4.73 (4.17, 6.27)	0.10 - 5.00
Interferon-γ (pg/ml)	3.48 (2.82, 3.93)	0.10 - 18.00
Tumor necrosis factor-α (pg/ml)	3.68 (2.83, 4.95)	0.10 - 23.00
$CD4^+$ T cells (%)	46.36 (34.32, 51.48)	25.34 - 51.37
$CD8^+$ T cells (%)	20.84 (18.49, 26.52)	14.23 - 38.95
$CD4^+$ / $CD8^+$ ratio	2.04 (1.58, 2.67)	0.41 - 2.72
D-dimer (μg/ml)	0.85 (0.36, 1.79)	< 0.5
Fibrinogen (g/L)	4.29 (2.88, 5.71)	2.00 - 4.00

*HbA1c was done only in patients with diabetes.
Abbreviations: eGFR: estimated glomerular filtration rate; ESR: erythrocyte sedimentation rate.

3. Results

3.1. The demographic and clinical characteristics

The clinical and demographic data of all patients were depicted as the median and interquartile range in brackets in Table 1 and the serological data in the same format were shown in Table 2.

The median age of infected patients was 57.50 years old, ranging from 29 to 76 years old as shown in Table 1. All patients had a fever before hospitalization (maximum temperature: 39.00 (38.60, 39.23) °C), while patients had slightly increased pulse frequency (96.00 (84.25, 115.50) beats per minute), normal respiratory frequency (20.00 (18.75, 22.00) times per minute) and normal blood oxygen saturation (96.50 (93.50, 99.00) %) when admitted. The median interval time between the emerge of the respiratory symptoms / fever and the date of confirmed diagnosis of NCP was 11.00 (8.25, 17.00) days. None of the patients had medical history of tumor, heart failure, cerebrovascular disease, kidney disease, or nursing house stay. Five out of ten patients had diabetes, two had hypertension and two had Type B hepatitis. As displayed, their blood pressure (systolic blood pressure: 127.50 (115.75, 139.25) mmHg; diastolic blood pressure: 90.50 (69.25, 96.00) mmHg) and liver function (Total bilirubin: 12.75 (8.23, 20.73) μmol/L; Alanine aminotransferase: 36.50 (26.25, 62.50) u/L) were well-controlled. However, the blood glucose level was elevated (HbA1c in diabetic patients: 8.80 (6.70, 9.35) %). In addition, d-dimer and fibrinogen were increased, suggesting hypercoagulation and hyperfibrinolysis in these patients. Neither acute renal dysfunction nor myocardial injury was found in these ten patients (serum creatinine: 88.00 (69.75, 92.75) μmol/L; troponin: 2.25 (1.55, 5.63) ng/L).

As to the inflammatory indicators, white blood cell (4.94 (4.06, 5.47) $\times 10^9$/L) and lymph cell counts (1.43 (0.57, 1.79) $\times 10^9$/L) were both within normal range although the lymph cell counts were relatively lower. C-reaction protein (CRP) (3.28 (1.14, 33.33) mg/L) and erythrocyte sedimentation rate (ESR, 22.50 (8.00, 78.50) mm/h) increased as compared to the normal reference whereas procalcitonin (0.075 (0.065, 0.105) ng/ml) was normal. Likewise, interleukin-6 (IL-6) (6.49 (4.96, 21.09) pg/ml) increased while other humoral immune factors, including IL-2, IL-4, IL-10, interferon-γ, and tumor necrosis factor-α were within normal range. The average ratio of $CD4^+$ cells to $CD8^+$ cells (2.04 (1.58, 2.67)) was slightly raised but still within the range.

All patients revealed either unilateral or bilateral ground-glass opacity in lung CT scan. Only one of the patients who had chronic obstructive pulmonary disease (COPD) presented suspicious unilateral pleural effusion.

3.2. The absence of SARS-CoV-2 in EPS and its relevance to nasopharyngeal RNA and serum immunoglobins

The patients were stratified into two groups subsequently. The first group was the patients who had positive nasopharyngeal swabbing SARS-CoV-2 within 3 days of the EPS taken day (PNS group, n = 3) and the other group was the patients who had positive nasopharyngeal swabbing SARS-CoV-2 before admission but turned negative few days before the EPS taken day (PNNS group, n = 7).

Although nasopharyngeal swabbing SARS-CoV-2 RNA had been reported positive as a medical history for all patients before admitted, it turned negative within seven patients in the nasopharyngeal swabbing re-tests before the EPS was taken. Only three out of ten patients had positive swabbing nasopharyngeal SARS-CoV-2 RNA within 3 days.

Table 3
The SARS-CoV-2 in EPS and its relevance to nasopharyngeal RNA and serum immunoglobins.

Patient No.	SARS-CoV-2 RNA in urine pre-palpation	SARS-CoV-2 RNA in EPS post-palpation	Nasopharyngeal SARS-CoV-2 RNA within 3 days of prostate palpation	Serum SARS-CoV-2 IgM	Serum SARS-CoV-2 IgG	The interval (days)*
1	Negative	Negative	Positive	Positive	Positive	NA
2	Negative	Negative	Positive	Positive	Positive	NA
3	Negative	Negative	Positive	Positive#		NA
4	Negative	Negative	Negative	Negative	Positive	23
5	Negative	Negative	Negative	Negative	Positive	29
6	Negative	Negative	Negative	Negative	Positive	14
7	Negative	Negative	Negative	Negative	Positive	3
8	Negative	Negative	Negative	Negative	Positive	18
9	Negative	Negative	Negative	Negative	Positive	8
10	Negative	Negative	Negative	Positive#		20

*The interval between the first negative nasopharyngeal SARS-CoV-2 RNA test and the day that EPS was taken.
Immunoglobin was detected unspecificly regardless of IgM or IgG because of the shortage for specific testing kits.
EPS: expressed prostatic secretion.
NA: not applicable.

Therefore, the interval between the latest negative SARS-CoV-2 RNA test and the day that EPS was taken was counted for the previous seven patients. The longest interval was 29 days while the latest was 3 days before the EPS test as shown in Table 3.

Serum SARS-CoV-2 IgM and IgG were also tested qualitatively during hospitalization (Table 3). Unfortunately, two patients had unspecific tests for SARS-CoV-2 immunoglobin because of the shortage for specific testing kit (shown in Table 3). There were two patients with positive nasopharyngeal SARS-CoV-2 RNA manifested positive serum IgM, while all patients had positive serum IgG (Table 3).

Unexpectedly but fortunately, none of the patients had positive COVID-19 RNA in EPS regardless of nasopharyngeal SARS-CoV-2 RNA.

4. Discussion

All patients in our study had a fever, maximum at 39.6℃, before hospitalization which supported the importance of temperature test in public area. Our data also presented increased inflammatory indicators, i.e. CRP, ESR, and IL-6 (Table 1), which has also been previously proved by other studies [15,16]. In our study, serum IgM against SARS-CoV-2 was only positive for patients in the PNS group indicating a recent infection. At present, there is no evidence for how long IgM will sustain. However, our data to some extent signified that serum IgM could be a potential and safer surrogate for nasopharyngeal swabbing SARS-CoV-2 RNA as a recent infectious indicator.

It has been raised concerns previously with respect to the male reproductive system during COVID-19 infection since the virus was verified to be related to testis damage [17]. Sexual transmission of COVID-19 has also been concerned since another pandemic pathogen, Ebola virus, was detected in semen and contagious in-between sexual partners [18]. The semen sample was still positive for Ebola virus at 565 days after discharge [18].

The EPS is secreted by prostate and is an essential component of semen, accounting for one tenth to one third volume of the ejaculation. Prostate fluid protects and nourishes the sperm cells. It would be emphasized that the EPS was negative for the SARS-CoV-2 RNA test according to our data, notwithstanding it was supposed that the prostate was one of the most susceptible organs. The SARS-CoV-2 RNA in EPS was not only negative in the PNNS group, but also negative in the PNS group. This strongly suggested the absence of SARS-CoV-2 in prostate, which possibly implied no prostate injury and the safety of the sexual behavior during COVID-19. In keeping with our data, several studies published very recently have demonstrated the negativity of SARS-CoV-2 in semen sample [11,12,19] although *in vitro* study had proposed the susceptibility of testis [17]. On the contrary, another recent publication reported that six COVID-19 patients had positive SARS-CoV-2 in semen samples [20]. However, the method description on the obtaining of semen samples in this publication were not fully described. Even so, this result makes the sexual transmission of SARS-CoV-2 more ambiguous. Given the fact that EPS is an important part of the semen, our results utterly supports these previous negative findings. However, it is worthy to mention that the EPS in our study independently verified the negativity of SARS-CoV-2 in the prostate which is different from semen sample in the previous findings.

Nonetheless, it would be prudent to recognize the false negative results in SARS-CoV-2 RNA test although the false negative results were more common in the nasopharyngeal swabbing RNA test in terms of non-standard swabbing operation. The potentially high false negative in the nasopharyngeal swabbing test has been raised concerns of the clinicians lately [21] since there were patients had a “turn positive” of COVID-CoV-2 but actually had a false negative test previously [22]. Consequently, RNA tests in repetition are needed both for the nasopharyngeal swabbing and for the EPS test in the future to confirm the true negative. In addition, the relatively small sample size is a major limitation of our study in the light of the strict inclusion criteria. A larger cohort is warranted to confirm the absence of sexual transmission during COVID-19. The copy number of viruses also needs to be assessed in parallel, while Meares-Stamey 4-glass Test might also need to be operated instead of two glass tests in the future study. Another limitation of the study is the no sampling of the seminal fluid. A study with both EPS and seminal fluid is therefore called for confirming the absence of SARS-CoV-2.

Considering what we have studied is only a tip of the iceberg, more collative actions and scientific evidences are needed to turn this pandemic.

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Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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