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REVIEW ARTICLE

Persistence of SARS-COV-2 in body fluids: a bystander or whistle blower

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ABSTRACT

The novel Coronavirus COVID-19 is wrecking a havoc across the globe and has been declared as a pandemic by WHO. Apart from transmission and shedding of the virus through respiratory secretions in the form of droplets (mainly), several studies have shown the presence of the virus in various samples such as stool, urine and occasionally in blood, semen, tears and breastmilk. Whereas government authority guidelines consider a person as cured from COVID-19 when along with clinical improvement no more virus can be detected primarily on respiratory samples along with clinical improvement; the persistence of the virus in these body fluids even after clinical recovery and negative RT-PCR test results on respiratory samples, has raised many questions about the elusive nature of this novel virus along with the possibility of other routes of transmission of this virus in the community. Although studies performed till now across the globe on persistence of SARS-COV-2 in various body fluids are sparse, in this review we would like to present and analyse the results of those studies performed globally on the aforesaid topic to get a better insight of this side of the COVID-19 story.

Keywords: COVID19; Coronavirus; Body fluid; Viremia

INTRODUCTION

Towards the end of 2019, the world witnessed a sudden emergence of pneumonia-like-illness in the Wuhan city (Hubei Province) of China with much speculation, the cases were attributable to a new corona virus named SARS-COV-2 (formerly 2019-nCoV). In no time it spread across the globe as a pandemic affecting almost the entire world and posing a major public health threat. It was declared as a pandemic by WHO on 11th March, 2020 (1).

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Respiratory droplets through person-to person close contact is one of the prime mode of transmis- sion of SARS-COV-2. Real-time reverse transcrip- tion polymerase chain reaction (RT-PCR) testing of respiratory specimens for SARS-COV-2 RNA is currently the reference standard for diagnosis, and is also being used as guidance for patient isolation /quarantine and/or hospital discharge. Other specimens like blood, faeces, urine and tears have also been tested as an alternative samples for the detection of viral RNA (2). In this article we tried to analyse the results of various samples other than respiratory for diagnosis as well as persistence of SARS-COV-2 in various studies performed worldwide till the end of June 2020.

The dilemma of viral clearance from body fluids. The percentage positivity rates in different body

fluids were studied by various authors. The positivity was found to be highest in Bronchoalveolar lavage (BAL) fluid (93%). The positivity rate from sputum and nasal swab sample was around 72% and 62% respectively. These are significant when compared to the rate of positivity for SARS-COV-2 from various other samples such as fibro-bronchoscope brush biopsy (46%), pharyngeal swabs (32%), faeces (29%) and blood (1%) (3). In India, as per the MOHFW guidelines, it is considered as a documented evidence of viral clearance when at least two upper respiratory tract samples from a COVID-19 patient for SARS-COV-2, collected at ≥ 24-hour intervals are negative. But the discharge policy for the same is different and is modified from time to time based on the prevailing scenarios (4).

The presence of SARS-COV-2 viral RNA in various body fluids, secretions, and excreta of a patient of COVID-19, might reflect the infectivity of the patient which is still a topic of debate and research. There have been studies to show that virus shedding is persistent in other body fluids where risk of viral transmission was there even after nasopharyngeal swabs were negative. It is still a matter of research to decode the fundamental pathophysiology related to the persistence of SARS-COV-2 in various body fluids of a patient of COVID-19 along with the mechanism and process of clearance of viral RNA, which is mostly dependent on immunobiology of SARS-COV-2 as per the existing knowledge on this topic. In a study by Long et al. it was found that the median duration of SARS-COV-2 shedding was 19 days (interquartile range (IQR), 15-26 d) among the 37 asymptomatic SARS-COV-2 positive patients which was longer than viral shedding among the symptomatic group. However, there are several factors which might contribute to the variation of duration of viral shedding which include the severity of disease, frequency of specimen collection and type of samples. But this outlook of the virus shedding cannot be also overlooked (5).

SARS-COV-2 shedding through gastrointestinal tract. Gastrointestinal involvement is variable and seen in around 2-35% of patients (6) infected with SARS-COV-2. Studies showed that patients with gastrointestinal symptoms had a longer duration of viral clearance from the onset of symptoms (P<0.001) and had higher faecal virus positivity than those with respiratory symptoms (73.3% vs 14.3% and P= 0.033) (7). Zheng et al. in his study, showed that in patients with severe illness, the median virus shedding duration in the respiratory samples was 21 days (14-30 days) which was significantly longer than in mild disease where it was 14 days, (10-21 days and P=0.04). The median duration of virus in stool was 22 days (17-31 days) which was significantly longer than respiratory samples where it was 18 days (13-29 days; P=0.02) and serum samples was 16 days, (11-21 days; P<0.001). Hence it was seen that viral load was dependent on the sample type, with highest load in respiratory samples, followed by stool samples, and the lowest being in serum samples. Patients with severe disease had higher viral loads in respiratory samples, than those with milder form. However there was no significant difference in viral load between patients with mild disease and severe disease in stool and serum samples (8). Ling et al. in their study found that among 66 successfully recovered patients, SARS-COV-2 viral RNA was still positive in the stool specimens taken from 11 convalescent patients (16.7%). The rest 55 patients were tested negative for SARS-COV-2 in stool samples by RT-PCR, with a median duration of 11 days (9-6 days). However, it was observed that 12 out of those 55 (21.8%) patients were negative for stool as well as oropharyngeal swabs at the same time and 43 (78.2%) had a longer duration with a median delay of 2 days (1-4) days until stool specimens were negative for viral RNA than for throat swabs, with a median delay of 2.0 (1.0-4.0) days. However, persistence of viral RNA was noted in the urine samples of 3 out of 4 patients, even after negative throat swabs (9).

According to the study by Ling et al. stool and sputum samples of 22 patients remained SARS-COV-2 RNA positive up to 13 and 39 days respectively, following negative oropharyngeal samples; which indicates the delayed clearance of viral RNA in patients' stools and sputum samples. It is believed that CD4+T lymphocyte count might be predicting viral shedding duration in stool of infected patients and therefore it may be pertinent to identify viral RNA in these samples during convalescence (9).

Interestingly the observation of Xu et al. on 10 paediatric SARS-COV-2 patients revealed that rectal swabs were positive in 8 patients even after negativity in nasopharyngeal swabs two of the four patients became positive in their rectal swabs later even after the two consecutive rectal swabs and nasopharyngeal swabs were initially negative. However, they

could not find any replication-competent virus in faecal swabs, which may be a requirement to confirm the potential for faeco-oral transmission (10).

Among the 15 patients studied by Zhang et al. 80% (8/10) were positive in oral swab on day 0 of testing, however on day 5 anal swab positivity were more 75% (6/8) than oral swab positivity 50% (4/8). It is being hypothesised that with the passage of days post infection, there is a shifting of RNA positivity from oral swabs to anal swabs. However the bearing on the faecal positivity for prolonged periods beyond oral positivity needs to be assessed in terms of assessing the infectivity status of the patient or merely as shedding of RNA without much community transmission (11).

Wölfe et al. observed that in three out of eight patients, the concentration of viral RNA in throat samples peaked during the first week of symptoms and thereafter there is a gradual decline. Moreover, the viral RNA load was also high in stool samples. They found out that the course of the viral RNA concentration in both throat samples and stool were similar except in one case where excretion of the virus was of different pattern than that of the throat samples suggesting independent replication in the intestinal tract. However, in this patient although the symptoms waned at end of the first week but viral RNA remained detectable in throat swabs until second week. It was also noted that even after complete resolution of clinical symptoms, stool and sputum samples remained RNA-positive over three weeks in six of the nine patients (12). Wölfe et al. also mentioned that although viral RNA was detected from stool samples, however; isolation of virus was not fruitful from the 13 stool samples taken from four patients between day 6 and day 12. They suggested that the isolation of virus depends on the viral load, and isolation was not possible in samples with a viral load less than 10^6 copies per ml (12).

Goh et al. in his study mentioned that SARS-COV-2 is armed with a more harder outer shell compared to SARS-COV-2 corona virus which offers considerable resistance to the action of enzymes present in saliva and mucus. Therefore, there is persistence of SARS-COV- 2 in saliva and mucus with a higher viral load. This could be a plausible cause of presence of the virus in stool of patients infected with SARS-COV-2. Moreover, it is observed that patients having gastro intestinal symptoms like diarrhoea etc might prevent the virus from staying too long in the different areas of gastrointestinal track, thereby withstanding antimicrobial enzyme and digestive juice could possibly lead to the stage of persistent shedding of the virus in stool of these patients (13). However, more research has to be made to come to a final conclusion on this point.

Angiotensin-converting enzyme-2 expression (specific cell receptor) and host cellular trans-membrane serine protease (TMPRSS) are essential for virus entry into cells and both are present in absorptive enterocytes as well as upper epithelial cells of oesophagus besides lung cells (14). There may be shedding of SARS-COV-2 through stool in at least a subset of patients and presence of viable and infectious SARS-COV-2 virions, (which is of major concern related to its infectivity) can't be ruled out just by the mere detection of viral genetic material in faecal matter of those patients. Interestingly, in the study by Wang et al. where they found live SARS-COV2 virions in the stool of two patients without any Gastrointestinal symptoms may suggest another route for transmission of the virus apart from normal respiratory route and this might contribute towards faecal shedding of SARS-COV-2 in the community. The persistence of the virus in stool for longer period necessitates further insight before considering a person them non-infectious. However, more studies are required to indicate the spread of SARS-COV-2 through faeco-oral route or change policy of labelling patients non-infectious based on negativity in respiratory samples (3).

Persistence of SARS-COV-2 in other body fluids

Blood. The presence of SARS-COV-2 in blood has also been documented in some studies. In a study by Wang et al. the virus showed positivity in blood among 1% of the cases, and hence its detection in blood has been a matter of concern during blood transfusion (3). In another study among the blood transfusion donors in real-time as well as retrospectively, it was found that plasma samples were positive for SARS-COV-2 RNA from 4 asymptomatic donors. This was detected during routine screening of blood donors, who was considered as a healthy population. However, the virions in blood or whether the virus could be transmitted through blood products, is not clear but the potential risk should not be neglected (15).

Saliva. Epithelial cells of salivary gland in a study

by Lee et al. showed elevated expression of ACE 2 receptors which are critical receptor for COVID-19. Hence the salivary gland may act as reservoir of SARS-COV-2 virus and shedding through saliva can occur (16). In a study by Azz et al. among the 25 COVID-19 positive patients all of them showed positive of SARS-COV-2 in saliva (17). In another study by Kelvin Kai-Wang To et al. SARS-COV-2 was detected in the saliva of 91.7% (11/12) of patients. It was seen that serial saliva viral load monitoring done in 6 patients showed a declining trend. However it was observed that 33 patients whose nasopharyngeal specimens tested negative for SARS-COV-2, all the saliva specimens of them were also negative (18).

Fluid of nasolacrimal system: Tears. Transmission of SARS-COV-2 through infected ocular tissue or fluid is still a matter of debate. The nasolacrimal system is believed to act as a conduit for passage of virions from the upper respiratory tract to the ocular system and hence may represent a potential source of SARS-COV-2 transmission (19). So far as shedding of the virus in tears is concerned Xia et al. showed that among 30 COVID-19 confirmed patients, only one patient had conjunctivitis and his tear samples was positive for viral RNA by RT PCR. Rest of the 29 samples tested negative (20). Similarly in a study by Jun et al. among 17 COVID19 patients, none of the tear samples grew SARS-COV-2 on viral isolation and no RNA for SARS-COV-2 was detected by RT-PCR although only one patient had mild ocular symptoms during the course of illness (10). Considering these findings from limited studies, we can assume that the risk of SARS-COV-2 transmission through tears is extremely low but this fact should not be neglected considering the illusive nature of SARS-COV-2 transmission.

Seminal and vaginal fluid. In a study performed by Li et al. on seminal samples for persistence of SARS-COV-2 with 38 participants (23 clinically recovered and 15 acute stage of infection), 6 (15.8%) were positive and among these positive patients, 26.7% (4/15) were in acute stage and 8.7% (2/23) were in clinically recovered stage. As 8.7% (2/23) of patients were still shedding the virus in the semen after clinical recovery phase, there is a remote possibility of sexual transmission of SARS-COV-2 through seminal fluid (21). On the other hand, in a study conducted by Qui et al. in 10 vaginal fluid samples of COVID 19 positive patients, no virus was isolated from these vaginal samples. Hence, shedding of SARS-COV-2 virus in vaginal fluid still questionable. More studies with bigger sample size involving patients in different phases of illness is required to prove or disprove these findings (22).

Urine. The shedding of the SARS-COV-2 through urine was first published by Peng et al. where it was observed that out of the 7 COVID-19 positive patients, one patient was positive for SARS-COV-2 in urine on day 7 however the patient did not show any urinary symptoms (23). In a study performed by Sun et al. SARS-COV-2 RNA was isolated from urine sample of a 72 year old man on day 12 post infection (February 5th) for the first time. It was also noted that this person showed periodically positive results in RT–PCR test for 42 days (March 6) even after disappearance of symptoms. Hence it is a bit difficult to comment on the infection of genitourinary track of this patient with SARS-COV-2, but the isolation of infectious SARS-COV-2 in urine sample raises the possibility of fecal/urine-respiratory transmission of the virus (24).

Breast milk. The shedding of SARS-COV-2 in breast milk is also documented in a study by Grod et al. where the virus was isolated from the breast milk of one of the two mothers for 4 consecutive days which coincided with mild COVID 19 symptoms. The throat swab for both the babies were also positive during that period. However, so far there is inadequate data regarding transmission of virus via breastfeeding and further studies are required to elucidate this (25).

CONCLUSION

In the context of present pandemic of SARS-COV-2, the respiratory route of transmission is considered to be the most efficacious route of transmission of the disease. Moreover, most of the laboratories as well as the RT-PCR kits and diagnostic guidelines are designed and has been validated for detection of the virus in the respiratory samples. Even the consideration of a patient being treated and recovered from COVID-19 infection is also based on the resolution of respiratory symptoms and clearance of virus from these respiratory samples. But the persistence of vi-

rus in various body fluids of SARS-COV-2 patients such as faeces, tears, breast milk and semen even after clinical recovery raise an important question on modes of transmissibility as well as the duration of infectivity of these patients. Being a novel virus most of the facts are still elusive for us and the finding of various studies related to persistence of the virus in these body fluids other than respiratory secretions mentioned in this review may pave a path for more accurately designed studies involving a significant number of patients in different healthcare settings.

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