

Review Article

SARS-CoV-2: Prolonged Viral Shedding and Persistent PCR Positivity: A Case Study from Pakistan

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Abstract

Introduction: Prolonged viral persistency and shedding are the major concerns associated with emerging variants of SARS-CoV-2. Complete viruses and viral fragments may persist for unusual and longer periods in both symptomatic and non-symptomatic patients.

Case presentation: In this study, we have reported unusual persistency of SARS-CoV-2 in 26 years old young patient from Islamabad, Pakistan.

Conclusion: In conclusion, to avoid viral persistency for a long duration, precise treatment and immune-boosting therapies must be recommended. Further, nontherapeutic interventions and preventive measures are necessary to avoid viral transmission and possible reinfection.

Keywords: COVID-19, Immunocompromised, Persistency, Viral shedding

Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the causative agent of Coronavirus disease 2019 (COVID-19) is a major concern of twenty first century [1]. This detrimental zoonotic and highly transmissible virus is responsible for the ongoing pandemic and poses threats to public health globally [2]. Continuous efforts revealed the epidemiology, pathogenicity, presiding results, genomic sequences, and precise diagnostic approaches of SARS-CoV-2 infections. Despite these facts, the burden of pandemic is still increasing due to the emerging variants[3,4], microbial coinfections [5], recrudescence [6], reinfections [7], prolonged viral shedding [8] and persistent RNA positivity [9] (Table 1).

Recently, patients with COVID-19 revealed persistently positive SARS-CoV-2 nucleic acid test results despite resolved clinical symptoms have attracted a lot of attention [10]. Researchers assumed long-term persistency of virus in the human body even after apparent recovery or negative results of nasopharyngeal specimens via PCR. Viral load appeared to be higher in the upper respiratory tract within the first week after symptom onset, and later in the lower respiratory tract. Viral shedding and viral load are important determinants of disease progression and transmission [11].

Table 1: Definitions.

Terms	Descriptions
Persistency	Unusual and prolonged presence of viral RNA in the body fluids.
Re-positivity	Detecting the presence of viral RNA following negative RT-PCR tests up to 90 days.
Recrudescence	Reactivation/ relapse of infection after clinical improvement within 90 days of first infection due to persisting viral fragments.
Reinfection	Infection caused by same or phylogenetically distinct respiratory and non-respiratory viruses after natural immunity and or vaccination.

In this study, we reported a ruled-out case of SARS-CoV-2 in young male patient of 26 years from Islamabad Pakistan, with extremely prolonged and persistent viral shedding for six weeks.

Case Presentation

A young student of 26 years with past medical history of respiratory tract problems including pulmonary tuberculosis, recovered in 2019 with COVID-19 symptoms including cough, fatigue, body pain and mild dyspnea introduced for the diagnosis of SARS-CoV-2 infection. The RT-PCR of nasopharyngeal samples were tested positive initially on April 20, 2021. Self-isolation was suggested with symptoms resolving treatment (paracetamol). The test was performed after RNA extraction (Qiagen Viral RNA Mini Kit) on ABI 7500 Real-Time PCR detection system with internal and external positive controls via the SARS-CoV-2 detection protocols.

After initial diagnosis, the patient was again COVID-19 positive after five days i.e. on April 25, 2021. At this stage, no clinical or physical examinations were performed while antibiotic treatment was recommended for five days. The symptoms were resolved during first week of antibiotic treatment. On May 18, 2021, persistency for SARS-CoV-2 with altered and mild symptoms were observed again. The major symptoms were anosmia and dyspnea (Figure 1 - Case timeline). Empirical antibiotic treatment was recommended to overcome the possible health problems i.e. Azithromycin 500 mg/day with two-fold increase compared to initial dose of 250 mg/day.

To investigate the patients' health status and immune response, recommended biochemical tests were performed on 33rd day of infection i.e. on May 22, 2021. Anti-SARS-CoV-2 antibody test was performed on Cobas e411 analyzer (Diagnostic Roche), a fully automated instrument employing Electro Chemiluminescence (ECL)

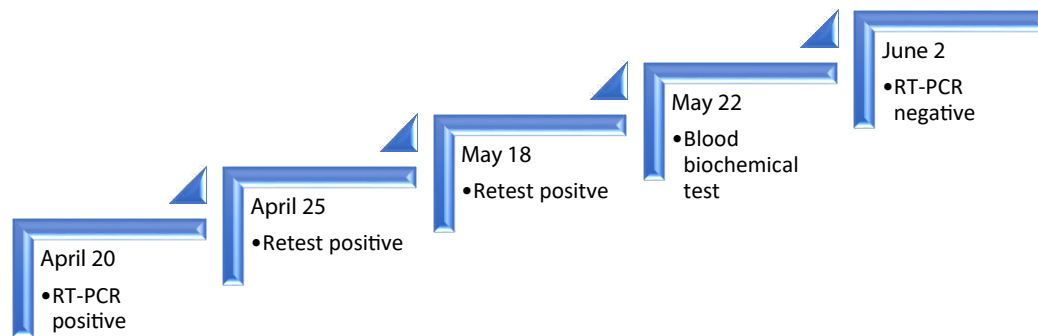


Figure 1: Case timeline of patient 1 (male, age 26 years).

Table 2: Biochemical tests.

Tests	Value	Reference value	Interpretation
Ferritin	160.9 ng/ml	14-250	Normal
CRP	0.13 mg/l	0.00-10.00	Normal
FDPs (D-Dimer)	0.1 mg/l	0.00-0.50	Normal
Antibodies	15.13 COI	Cut off 1.00	Reactive

Abbreviations: CRP: C-reactive proteins; FDPs: Fibrin Degradation Products, COI: Cut Off Index.

technology for immunoassay analysis using FDA approved kits in human serum and plasma, revealed development of SARS-CoV-2 antibodies (titer 15.13 cut of index COI > 1).

The health status was normal with mild morbidity as the biochemical markers associated with COVID-19 including Ferritin, C-reactive proteins (CRP) and D-dimers were normal (Table 2). Complete blood count revealed drop in neutrophil counts while lymphocytes level was high (Table 3). Fortunately, the patient was declared recovered according to general discharge criteria (Figure 2) [12] on 43rd day of primary infection.

Discussion

Viral load remains high in upper respiratory tract during first week of infection or onset of symptoms and tends to decrease with time. Virus median duration of shedding is 8 days post onset of symptoms and drops below 5% after 15.2 days post onset of symptoms [13]. However, recent studies demonstrated viral shedding for long duration in immune-compromised individuals [14] in both symptomatic and asymptomatic patients of older age [15]. The current study reported unexpected and persistent infection of SARS-CoV-2 in young patient of 26 years for more than six weeks. To our understanding, this is the first report addressing prolonged and persistent viral positivity from Pakistan.

RT-RNA based repeated RNA positivity was considered primary indication of persistent infection as documented in previous studies [16]. Persistent shedding was significantly associated with persistent dyspnea and anosmia. Detection of viral RNA and confirmed retest positivity for SARS-CoV-2 in recovered patients is clinical indication of prolonged viral persistency or relapse of infection [17]. Analgesics and Azithromycin were recommended to eradicate disease morbidity. In persistent COVID-19 treatment, azithromycin plus hydroxychloroquine were more efficient previously [18].

Table 3: Complete blood count.

Parameters (units)	Reference value	Value
White blood cells (TLC) ($10^9/L$)	4.00-11.00	5.8
Neutrophils (%)	50.00-70.00	40
Lymphocytes (%)	20.00-40.00	48
Monocytes (%)	3.00-12.00	10
Eosinophils (%)	0.50-5.00	02
Basophils (%)	0.00-1.00	0.0
Red blood cells, count (Mil/Cm)	4.00-6.00	5.02
Hemoglobin (Hb) (Gm/dl)	11.00-16.00	14.0
Hematocrit (PCV) (%)	37.00-54.00	46.5
MCV (fL)	80.00-100.00	93.0
MCH (Pgm)	27.00-34.00	27.8
MCHC (g/L)	30.00-36.00	30.0
Platelets ($10^9/L$)	150.00-450.00	333
MPV (fL)	7.00-11.00	7.5
PDW (fL)	8.30-25.00	16.2
Procalcitonin (mL/L)	1.08-2.82	1.82

Abbreviations: TLC: Total Leucocytes Counts, PCV: Packed Cells Volume, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, MPV: Mean Platelet Volume, PDW: Platelet Distribution Width.

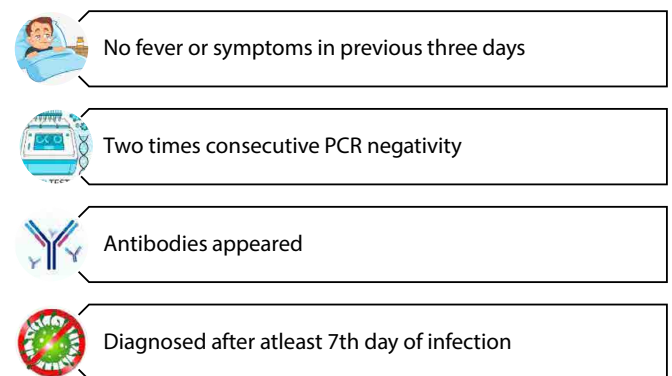


Figure 2: Criteria of discharge/declaring recovered.

In contrast to our findings, a study reported SARS-CoV-2 persistency for 59 days in young female patient with the age of 25 years [16]. The patient recovered without any antibiotic and antiviral

therapy as no consequences were observed with health issues and underlying morbidities. Another case report from Thailand revealed prolonged SARS-CoV-2 shedding in asymptomatic patient (age 30 years) for 110 days [15]. Symptoms resolution with shedding duration or asymptomatic patient could promote disease transmission and hence asymptomatic virus carriers can be still infectious [19]. Therefore, there is need of monitoring the surroundings of patients to prevent the risk of viral transmission.

Immunodeficiency plays a major role in prolonged viral shedding that can be observed even in asymptomatic individuals with weak immune system [14,20]. We demonstrated that delayed treatment, low dose medication, previous medical history, higher susceptibility due to weak immune system were responsible for persistent viral infection. Similarly, viral clearance in COVID-19 patients varies and delayed in patients with older age, multiple re-exposure, underlying comorbidities such as diabetes [21] respective therapies [22] and myeloma [23] etc. (Figure 3).

The worldwide discharge criteria (Figure 2) are improved clinical symptoms and two PCR negative test, needs modification for accurate and precise diagnosis [12]. Various studies emphasize precise and accurate decision of prolonged viral shedding and true infection. Confirmation and differentiating these hurdles might provide an insight of infection and beneficial support to the physician while treating the victim [24].

In addition, the patients experiencing only mild symptoms with extreme infection develops a weaker immune response which might explain predisposition to the reinfection [25]. Prolonged viral shedding and its particles poses diagnostic challenges. It might complicate infection control, treatment and might significantly contribute its role in morbidity and mortality associated with COVID-19 [26].

We declared patient recovered based on general discharge criteria (Figure 2). Laboratory biochemical tests, development of antibodies and clearance of symptoms are major factors which helped declaring recovery of patient from this regime. In summary, the possibility of prolonged viral shedding is significantly associated with health status of patient, timely diagnosis, and treatment strategies without any discrimination of age and gender. Further, patients recovered in long duration should be vaccinated after few weeks of recovery to prevent reinfection with emerging variants of SARS-CoV-2.

Conclusion

Comorbidities, immunodeficiency, unspecific drugs, drug discontinuation and waning immunity are the major contributors of prolonged and persistent viral infection. These regimes might further increase the susceptibility of patient for reinfection with various emerging variants. In this ruled out situation, the unusual persistency of SARS-CoV-2 and delayed viral clearance could be due to weak immune system, previous history of lung infection (tuberculosis) and drug discontinuation. In conclusion, to avoid viral persistency for long duration, precise treatment and immune boosting therapies must be recommended.

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Conflict of Interest

The authors declare no competing interest.

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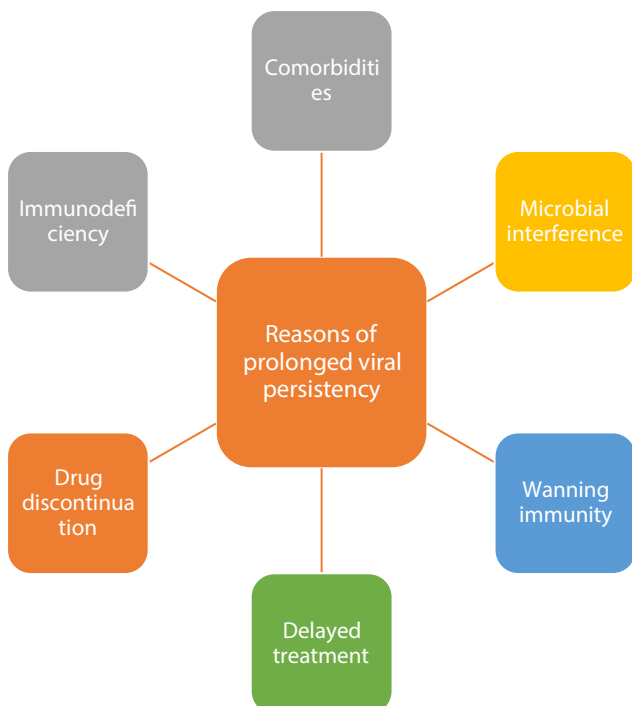


Figure 3: Major reasons of that can contribute to prolonged viral shedding (SARS-CoV-2).

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