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Prolonged persistence of SARS-CoV-2 in the upper respiratory tract of asymptomatic infected individuals

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Summary

Background: Duration of persistence of SARS-CoV-2 in the upper respiratory tract of infected individuals has important clinical and epidemiological implications.

Aim: We aimed to establish the duration and risk factors for persistence of SARS-CoV-2 in the upper respiratory tract of asymptomatic infected individuals.

Methods: Data of repeat rRT-PCR (real-time reverse transcription-polymerase chain reaction) test done for SARS-CoV-2 infected individuals at our institute at Jodhpur, India were analysed from 19 March to 21 May 2020. Duration of virus persistence was estimated with parametric regression models based on weibull, log-normal, log-logistic, gamma and generalized gamma distributions. Factors associated with prolonged viral persistence were analysed with the best-fitting model.

Results: Fifty-one SARS-CoV-2 infected individuals with repeat rRT-PCR test were identified with 44 asymptomatics. The asymptomatic individuals had median virus persistence duration of 8.87 days (95% CI: 7.65–10.27) and 95 percentile duration of 20.70 days (95% CI: 16.08–28.20). The overall median virus persistence including both symptomatic and asymptomatic individuals was found to be 9.18 days (95% CI: 8.04–10.48). Around one-fourth asymptomatics (10/44) demonstrated SARS-CoV-2 persistence beyond 2 weeks. Age <60 years and local transmission were found to be significantly associated with longer virus persistence among asymptomatic individuals on univariate regression but not in multivariate analysis.

Conclusion: Recommended home isolation duration for SARS-CoV-2 infected individuals in India should be extended from 17 days to at least 3 weeks. Prolonged persistence of SARS-CoV-2 in a considerable proportion of asymptomatic individuals merits attention with regard to ensuring universal infection prevention precautions irrespective of symptomatic status.

Introduction

As on 21 June 2020, COVID-19 has resulted in 8.7 million cases and 0.46 million deaths.[1] India has become the fourth most affected country worldwide with around 0.41 million confirmed COVID-19 cases.[1] The persistence of SARS-CoV-2 in body fluids has important clinical and epidemiological implications. A negative rRT-PCR (real-time reverse transcription-polymerase chain reaction) result had been considered as a surrogate marker of non-infectiousness of the individual. Consequently, it had been required for discharge of individuals undergoing treatment or hospital isolation in India until 8 May 2020, when testing prior to discharge had been discontinued under the revised strategy.[2,3] Also, the recommended duration of home isolation for both health workers and general public is being guided by expectation of viral negativity at the end of the isolation period.[3]

Materials and methods

Both oropharyngeal and nasopharyngeal swab specimens were taken from the individuals meeting suspect case definition for COVID-19 at our institute in Jodhpur, India.[4] Nucleic acid extraction and rRT-PCR for SARS-CoV-2 was done as per protocol approved by National Institute of Virology, Indian Council of Medical Research.[5] From among those found positive for SARS-CoV-2, duration of virus persistence was considered from the symptom onset date till the first negative rRT-PCR result. For asymptomatic individuals, the date of collection of first positive sample was taken instead of symptom onset. R software version 4.0.0 (with Survival and Flexsurv packages) was used for analysis of virus persistence duration with weibull, log-normal, log-logistic, gamma and generalized gamma models.[6] The duration estimates were taken from the best-fitting model based on minimum Akaike information criterion (AIC) value. Standard maximum likelihood approach was used to obtain the best model fit to actual data.

The SARS-CoV-2 infected individuals from Jodhpur district, India were classified as having local transmission. Individuals who had been evacuated from Iran in March 2020 and had been quarantined were classified as having transmission from abroad. Univariate and multivariate parametric regression was conducted with the best-fitting distribution for asymptomatic individuals with age, gender and type of transmission as co-variates. Interval and right censoring of duration of virus persistence were accounted for in the regression analysis.

Results

We analysed data of 9760 rRT-PCR tests conducted at our institute from 19 March to 21 May 2020, out of which 425 positive test results were obtained. Fifty-one SARS-COV-2 infected individuals had undergone repeat rRT-PCR testing after first positive test, out of which 44 were asymptomatic and 7 were symptomatic.

Around 80% (35/44) of the asymptomatic individuals were males (Table 1). Nearly one-fourth asymptomatic individuals (10/44) had virus persistence longer than 14 days. Maximum duration of shedding among them was 25 days (Supplementary file S1). The 10 asymptomatic individuals demonstrating local transmission were significantly younger than the 34 individuals who had transmission from abroad (*P* = 0.020 on unpaired *t*-test). The log-logistic model was found to be the best-fitting for estimating the duration of virus persistence (Table 2).

The median and 95 percentile durations of SARS-CoV-2 persistence in upper respiratory tract of asymptomatic individuals was found to be 8.87 days (95% CI: 7.65–10.27) and 20.70 days (95% CI: 16.08–28.20), respectively (Figure 1). While taking both the asymptomatic and symptomatic individuals together, the overall median and 95 percentile durations were found to be 9.18 days (95% CI: 8.04–10.48) and 20.92 days (95% CI: 16.61–27.55), respectively. The virus persistence duration of 10.98 days (95% CI: 8.38–14.44) for symptomatic individuals was not found to be significantly longer than that of asymptomatic individuals (*P* = 0.222).

Regression analysis for asymptomatic individuals was conducted with the best-fitting Log-logistic model. Upon univariate analysis, age <60 years and local transmission were found to be significantly associated with longer persistence of SARS-CoV-2 in the upper respiratory tract (Table 3). None of the co-variates were found to be significantly associated with prolonged virus persistence when age, gender and type of transmission were taken together in the multivariate model (Table 3).

Discussion

Prior to the introduction of home isolation strategy on 10 May 2020,[7] India had adopted the policy of universal health-facility-based isolation of all SARS-CoV-2 infected individuals irrespective of symptomatic status. This provided us with a unique opportunity to study the virus persistence among the asymptomatic individuals admitted at our institute.

Longer virus persistence has been demonstrated in severe illness as compared to mild COVID-19.[8] However, we did not find a statistically significant difference in virus persistence based on symptomatic status. Our finding of median duration of virus persistence of 9.18 days among all infected individuals was comparable to the estimate of 10 days as per laboratory surveillance data from India[3] and 10–12 days reported from China.[9,10] It was, however, lower than the 15 days reported from South Korea.[11] Considering only asymptomatic individuals, the median virus persistence of 8.87 days reported by us nearly matched that of 9 days reported from the *Diamond Princess* ship, indicating similar transmission dynamics.[12] The present study adds to the evidence of prolonged persistence of SARS-CoV-2 in upper respiratory tract in a considerable proportion of infected individuals.[8,9,11,13]

Association of local transmission with longer virus persistence on univariate analysis could have been confounded by the locally infected individuals being significantly younger than those evacuated from abroad. Possible association of younger age and variation in transmission pattern with prolonged virus persistence merits further exploration.

Although virus persistence measured through positive rRT-PCR of swab samples does not necessarily mean that the individual is infective, greater viral load indicated by lower cyclic threshold values correlates with cell culture infectivity.[13] Therefore, the finding of prolonged virus persistence even among asymptomatic individuals has important public health implications when large number of individuals with varying viral loads are considered in the community.

A study from the USA had recommended that people infected with SARS-CoV-2 should cease infection prevention precautions and should return to work only after 33 days of symptom onset or a negative test result.[14] Furthermore, the finding of prolonged and intermittent viral shedding also helps explain why earlier many 'recovered' patients had re-tested positive after being discharged from hospital.[15,16] These findings reset the expectation of viral shedding to a longer

Table 1. Baseline characteristics of the 44 asymptomatic SARS-CoV-2 infected individuals studied for the duration of virus persistence in upper respiratory tract

Characteristics	Local transmission N = 10	Transmission from abroad N = 34	Total N = 44
Age in years, mean (SD)	39.3 (23.4)	61.5 (10.3)	56.4 (17.1)
Gender, *n* (%)			
Male	8 (80.0)	27 (79.4)	35 (79.5)
Female	2 (20.0)	7 (20.6)	9 (20.5)

SD, standard deviation.

Table 2. Fit of parametric survival models for estimation of duration of virus persistence in upper respiratory tract of asymptomatic SARS-CoV-2 infected individuals (*n* = 44)

Type of distribution used in model	Minus 2 log likelihood value	No. of model parameters (k)	AIC value (−2 log likelihood +2k)
Weibull	250.15	2	254.15
Log-normal	244.22	2	248.22
Log-logistic	243.39	2	247.39
Gamma	245.90	2	249.90
Generalized gamma	244.22	3	250.22

AIC, Akaike information criterion.
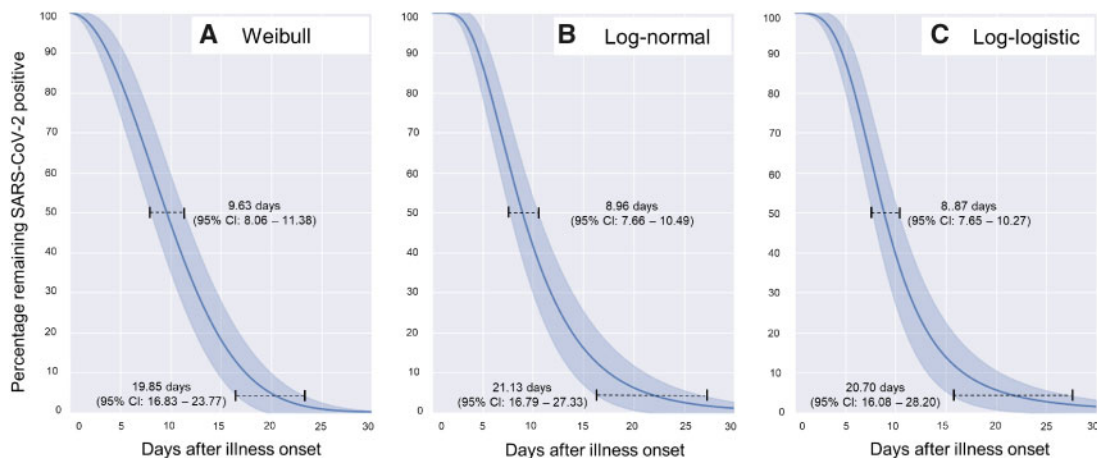
Figure 1. Duration of persistence of SARS-CoV-2 in the upper respiratory tract of asymptomatic individuals as fitted to (**A**) Weibull, (**B**) Log-normal and (**C**) Log-logistic distributions (*n* = 44)

Table 3. Log-logistic regression for plausible risk factors for prolonged duration of virus persistence in the upper respiratory tract of asymptomatic SARS-CoV-2 infected individuals (*n* = 44)

Co-variates	Exp (β) value (95 % CI)	P-value (Wald test)	AIC value for model
Univariate regression (one variable at a time)			
Age ≥60 years vs. <60 years	0.69 (0.52–0.92)	0.011	243.01
Male vs. female gender	0.71 (0.48–1.06)	0.097	246.80
Local transmission vs. transmission from abroad	1.48 (1.04–2.10)	0.029	244.56
Multivariate regression (all three variables together)			
Age	0.77 (0.57–1.03)	0.076	242.06
Gender	0.72 (0.51–1.00)	0.051	
Type of transmission	1.28 (0.90–1.81)	0.171	

AIC, Akaike information criterion.

duration, especially for asymptomatic SARS-CoV-2 infected individuals.

Our estimates could be generalizable to settings wherein large proportion of infected individuals remain asymptomatic. We had the limitation that we could not separately estimate the duration of virus persistence in nasopharynx and oropharynx as both the swap tips were inserted together in the viral transport medium to increase the possibility of SARS-CoV-2 detection.

Conclusions

Based on our findings, a prolongation of current home isolation guidance from 17 days[7] to at least 21 days may be recommended in India. During the course of the COVID-19 pandemic, the emerging evidence has supported the role of asymptomatic individuals in transmission and prolonged viral shedding in considerable proportion of individuals.[17] This has prompted the WHO guidance to the general public on wearing masks in areas with suspected transmission, high population density or where physical distancing is not feasible.[18] The evidence of prolonged virus persistence among asymptomatic individuals in the present study further emphasizes the need for universal infection prevention precautions irrespective of symptomatic status.

Supplementary material

Supplementary material is available at *QJMED* online.

Authors' contributions

M.K.Garg conceived the idea of the study. S.S. extracted the data and S.S. and R.K. conducted the analysis. S.S. wrote the draft manuscript with further inputs from M.K.Garg, P.B. and M.K.Gupta. P.B. coordinated the data collection and V.L.N. coordinated the testing of samples. S.M. provided overall supervision of the testing, isolation, clinical care and research related to COVID-19 at AIIMS, Jodhpur, India. All authors approved the final manuscript.

Ethical approval

The study has been approved by the Institutional Ethics Committee of All India Institute of Medical Sciences (AIIMS), Jodhpur, India.

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