SARS-CoV-2 RT-PCR positivity in relation to clinical and demographic characteristics in residents of border quarantine centres, Khyber Pakhtunkhwa, Pakistan: a prospective cohort study

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Background: As international travellers were the primary source of sever acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, border checkpoints became an important tool to isolate cases. We determined the period prevalence and SARS-CoV-2 reverse transcription polymerase chain reaction positivity in relation to clinical and demographic characteristics in healthy travellers quarantined at the Pakistan–Afghanistan border.

Methods: The study was conducted from 15 to 25 April 2020. Period prevalence was calculated and the association between positivity and individuals' age, sex and occupation were assessed using χ^2 and Mantel–Haenszel tests. Logistic regression was used to calculate adjusted odds ratios (ORs) for each age group. Time-to-event (TTE) analysis was conducted to check the difference in positivity among various groups.

Results: In a total of 708 individuals, 71 tested positive (10%). Compared with those ≤20 y of age, the sex- and occupation-adjusted odds of testing positive were less among the older age group (41–60 y; OR 0.26, p=0.008). Taxi drivers had higher odds of testing positive (OR 4.08, p<0.001). Kaplan–Meier curves and hazard ratios (0.32, p<0.01) showed that the positivity period differed significantly across the pre-symptomatic vs asymptomatic group (26 vs 14 d).

Conclusions: The cases who were likely to acquire infection through occupational exposure largely remained asymptomatic. For effective control of transmission and the emergence of new variants, testing capacities should be revamped with effective isolation measures.

Keywords: asymptomatic infection, COVID-19, Pakistan, prevalence, SARS-CoV-2, transmission

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which cause coronavirus disease 2019 (COVID-19), was first detected in Wuhan, China, in December 2019.[1] By 28 June 2021, >180 million people were infected with SARS-CoV-2 and had caused 3.9 million deaths.[2] The rapid spread across the globe led to international border closures and travel bans. According to the Pew Research Center, by 1 April 2020, >9 in 10 people worldwide were living in countries with travel restrictions due to COVID-19.[3]

Travel bans may reduce the introduction and spread of SARS-CoV-2, but they are not without a social cost.[4] Citizens abroad could be trapped outside their home country and face significant challenges while returning to their home countries. As reported by the PEW Research Center in April 2020, 7.1 billion people (91% of the world's population) lived in countries whose borders were almost entirely closed for travellers.[3] Different countries have different screening protocols for new entrants on international borders. Some screen for fever and others conduct rapid or polymerase chain reaction (PCR) testing before departure or on arrival. However, such screening can miss a significant portion of infected individuals who are pre-symptomatic or asymptomatic.[5] Therefore many countries, including Pakistan, instituted quarantine, testing and isolation strategies.

Pakistan reported its first SARS-CoV-2 case on 26 February 2020—a traveller from Iran—almost 2 months after the first case in Wuhan, China.[6] Since then, the number of cases in Pakistan has surged to just under a million by the end of June 2021 and there is widespread community transmission throughout the country.[2] Pakistan closed all its land, sea and air borders on 13 March 2020 to reduce the risk of imported SARS-CoV-2 cases, followed by a series of strict lockdowns.[7] Currently, due to increasing fear about the spread of new COVID-19 variants, flight bans between different countries have become routine.

The objective of this study was to determine the period prevalence and SARS-CoV-2 reverse transcription (RT)-PCR positivity in all the healthy residents of four quarantine facilities in Khyber District. The analysis includes the association in relation to clinical and demographic characteristics of the residents. Initially all symptomatic individuals were excluded from the study. They were referred to the District Headquarters Hospital as per Health Department Khyber Pakhtunkhwa policies. Moreover, all healthy asymptomatic travellers were shifted to the quarantine centres for SARS-CoV-2 RT-PCR screening. To address the issue of pre-symptomatic infections, all positive individuals were followed in the quarantine centres for the development of symptoms. The individuals who were asymptomatic at the time of testing but developed symptoms afterwards were recategorized as pre-symptomatic for further analysis.

Methods

Study population

This study is based on 708 individuals who had no signs or symptoms of COVID-19 during the initial screening at the border checkpoints of Torkham in the Khyber Tribal District of Khyber Pakhtunkhwa province of Pakistan. Every inbound traveller coming into Pakistan through this border from 15 to 25 April 2020 was screened for symptoms, comorbidities, contact history, occupation and travel history to Iran or China with the help of a questionnaire. Individuals showing any signs or symptoms of illness were referred to the District Headquarters Hospital, Khyber, for further evaluation and were excluded from this study. The remaining asymptomatic individuals were kept in quarantine facilities established at four different locations in Khyber District for RT-PCR. These quarantine facilities received 708 asymptomatic and healthy individuals during the study period.

After an informed consent, nasopharyngeal and oropharyngeal swabs were taken from the study population and sent to the Public Health Reference Laboratory, Khyber Medical University for SARS-CoV-2 testing. The laboratory used SYSTAAQ 2019-Novel Coronavirus Multigene Real-Time PCR Kit (SYSTAAQ Diagnostic Products, Manassas, VA, USA)[8] to test the positivity of the samples for SARS-CoV-2. Following the World Health Organization (WHO) interim guidelines,[9] laboratory confirmation of COVID-19 was defined as a positive result for SARS-CoV-2 using an RT-PCR assay of nasal and pharyngeal swabs. To detect the positive test, the recommended cut-off cycle threshold value was taken as <32 cycles, for the above-mentioned test kit. If the samples were negative for both fluorescein amidites and hexachloro-fluorescein fluorescent dye probes, then they were run a second time and

if negative again, were declared as inconclusive or invalid. The testing of all 708 individuals was completed in 4 d and the results were received in 24–48 h after testing. The individuals who tested positive for COVID-19 were kept at the quarantine facilities, while the negative individuals were sent home. The positive individuals were followed for the development of any signs or symptoms to assess the possibility of pre-symptomatic infections. Each individual was subjectively and objectively assessed daily for fever, cough, shortness of breath, sore throat, rhinorrhoea, nausea, diarrhoea and headache. The individuals who were asymptomatic at the time of testing but developed symptoms afterwards were recategorized as pre-symptomatic. As depicted in Figure 1, the result of the first PCR showed that 596 (84%) were negative and 68 (10%) were positive. Furthermore, 44 (6%) of the tests were inconclusive. Nasopharyngeal swabs and RT-PCR of these 44 individuals who tested inconclusive were repeated, which resulted in an additional 3 positive individuals, 3 inconclusive and 38 negative. The 3 individuals with the persistent inconclusive result on repeat PCR were excluded from the study and analysis of the remaining 705 individuals is presented here.

At the end of the quarantine, 56 of 71 individuals tested negative for SARS-CoV-2 by RT-PCR and were discharged from the facility. Of the remaining 15 individuals, 13 tested positive and 2 had inconclusive test results. These individuals continued their quarantine until their test became negative. All the individuals tested negative by 21 May 2020 and were discharged from the facility.

Statistical analysis

We calculate the period prevalence using the entire asymptomatic population (i.e. 708 individuals). For further statistical analysis, we considered only the 705 individuals with a definitive result on an RT-PCR test. We stratified the prevalence by age group, sex and occupation. The cohort of infected individuals was followed for the entire quarantine period and monitored for the onset of COVID-19 symptoms to determine pre-symptomatic individuals. The proportion of those who developed symptoms afterwards (pre-symptomatic) was determined and compared by demographic factors. Mantel–Haenszel and χ^2 tests were used to determine if the symptom profile differed by age group, sex or occupation. Further, we estimated the odds of testing positive for each age group by adjusting for sex and occupation, considering the youngest age group (<20 y) as our base category.

Of 71 positive individuals, 13 tested positive at the end of the first quarantine and 2 had inconclusive test results. These 15 individuals were quarantined for a further 2 weeks. This prolonged viral load in some of the individuals prompted us to check if the duration of positivity and underlying prolonged viral load differed in our population. For this purpose we performed time-to-event (TTE) analysis using Kaplan–Meier curves and hazard ratios (Cox proportional hazards) by looking at the positivity period across sexes, age group, occupation and clinical features. We defined 'event' as testing negative for COVID-19 after first testing positive. The positive tested individuals were followed from their first RT-PCR test day until they tested negative at the end of their quarantine period. For inconclusive cases on the first test who tested positive on a repeat test, we assumed that they were positive but the test could not give results due to a technical error. Such cases were followed from the date of their first RT-PCR test. Similarly,
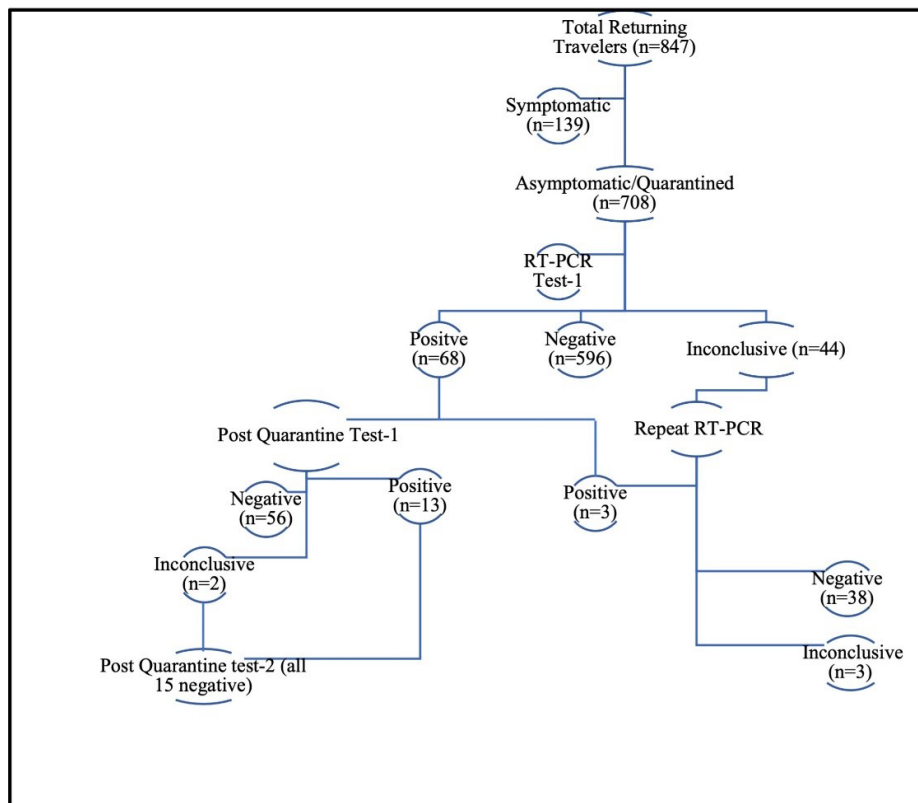
Figure 1. Flow diagram explaining the testing procedure for quarantined individuals.

individuals who first tested inconclusive but their repeat PCR was negative were assumed to be COVID-19 negative on the first test and were excluded from the analysis. Those who had inconclusive results for the first and repeat PCR tests (n=3) were censored on the date of their repeat PCR test. Those who tested negative on the first test were not included in the analysis, as they were not followed or kept in a quarantine centre.

All the analysis was conducted in R (version 4.0.3; R Foundation for Statistical Computing, Vienna, Austria)[10] using epiR,[11] survival and[12,13] survminer[14] and figures were produced using the package ggplot2.[15] Statistical significance was set at 0.05.

Ethical considerations

We used anonymized data for our study and permission to use the data for research purposes was obtained from the Office of the District Health Officer Khyber, Jamrud, Pakistan. The study was reviewed and approved by the Institutional Research and Ethical Board (IREB) of the Medical Teaching Institute Lady Reading Hospital, Peshawar, Pakistan (reference 1056).

Results

General characteristics

We present descriptive statistics in Table 1. Age ranged from 12 to 82 y and 527 (74.4%) were males. Our study population was

relatively younger, as 449 (64%) individuals were <40 years of age (Figure 2). The majority of our population were engaged in transport-related occupations such as drivers and conductors (166 [24%]), followed by housewives (146 [21%]) and business (133 [19%]). Ten percent of our study sample (71 individuals) tested positive, while the remaining 90% (634 individuals) were negative. Among the positive individuals, 65 (92%) were male, while only 6 (8%) were female. All the study population had travelled from Afghanistan, while none had a travel history to China or Iran.

Pre-symptomatic vs asymptomatic distribution

All 705 individuals were asymptomatic at the time of conducting the first PCR test. During quarantine, 15 (21%) of 71 positive patients developed symptoms an average of 3.3 d (range 0–8) after the first PCR test (Figure 3) and were considered as pre-symptomatic cases. The distribution of asymptomatic and pre-symptomatic cases in our population is presented in Table 2.

Fever and cough were the most commonly reported symptoms, followed by a sore throat and headache. A total of 7 (46%) of the patients also developed shortness of breath. Fourteen of 65 positive males (21.53%) and only 1 of 6 positive females (16.6%) developed symptoms. None of the individuals <20 y of age developed symptoms, while 10 (2.53%) developed symptoms in the age group 21–40 y, as shown in Table 2. The observed distribution for asymptomatic and pre-symptomatic cases did not differ

Table 1. Descriptive statistics of the study population

Variables	Overall (N=705)[a]	Negative (n=634)[a]	Positive (n=71)[a]	p-Value[b]
Age group (years), n (%)				<0.001
≤20	53 (7.5)	43 (6.8)	10 (14.1)	
21–40	396 (56.2)	347 (54.7)	49 (69)	
41–60	208 (29.5)	199 (31.4)	9 (12.7)	
≥61	48 (6.8)	45 (7.1)	3 (4.2)	
Sex, n (%)				<0.001
Male	527 (74.8)	462 (72.9)	65 (91.5)	
Female	178 (25.2)	172 (27.9)	6 (8.5)	
Occupation[c], n (%)				
Business/office related	133 (18.9)	126 (19.9)	7 (9.9)	
Elementary occupations	43 (6.1)	41 (6.5)	2 (2.8)	
Transport related	166 (23.5)	124 (19.6)	42 (59.2)	
Service, sales, teaching	53 (7.5)	50 (7.9)	3 (4.2)	
Agriculture	7 (1.0)	7 (1.1)	0 (0)	
Craft and trade worker	19 (2.7)	18 (2.8)	1 (1.4)	
Hospital	7 (1.0)	5 (0.8)	2 (2.8)	
Housewife	146 (20.7)	140 (22.1)	6 (8.5)	
Religious work	11 (1.6)	10 (1.6)	1 (1.4)	
No work	55 (7.8)	52 (8.2)	3 (4.2)	
Student	39 (5.5)	38 (6.0)	1 (1.4)	
Visitor	26 (3.7)	23 (3.6)	3 (4.2)	
Residence, n (%)				0.004
Peshawar	273 (38.7)	241 (38.0)	32 (45.1)	
Khyber	213 (30.2)	184 (29.0)	29 (40.8)	
Other	219 (31.1)	209 (33.0)	10 (14.1)	
Site of quarantine, n (%)				<0.001
Quarantine Centre Levis Centre Shahkas Jamrud	318 (45.1)	295 (46.5)	23 (32.5)	
Quarantine facility Boys Degree College Jamrud	197 (27.9)	192 (30.3)	5 (7.0)	
Quarantine facility Boys Government High School Landikotal	98 (13.9)	72 (11.4)	26 (36.6)	
Quarantine facility Girls Government High School Landikotal	92 (13.1)	75 (11.8)	17 (23.9)	

[a]Inconclusive cases are excluded.
[b]Fisher's exact test or Pearson's χ^2 test.
[c]Occupation categories include Transport (conductor, driver), Craft and trade worker (goldsmith, mechanic, tailor), Elementary occupations (chowkidar, labourer) and Service, sales, teaching (cook, shopkeeper, teacher).

across sex and age groups (i.e. Fisher's exact test was statistically non-significant).

Period prevalence and adjusted odds ratios (ORs)

The estimated prevalence of SARS-CoV-2 in our study population was 10% (71 of 708 individuals). As shown in our descriptive statistics, the distribution of positive cases varied across age groups. We found that positive individuals were predominantly male and belonged to relatively younger age groups. Therefore we estimated the sex, occupation and residence adjusted ORs for different age categories. As shown in Table 3, the odds of testing positive were lower among older age groups after adjusting for sex and occupation when compared with the <20 y group (21–40 y OR 0.49, p=0.079; 41–60 y OR 0.26, p=0.008; ≥61 y OR 0.44, p=0.3).

At the constant values for other terms, females had lower odds of testing positive than males; however, the results were not statistically significant (p=0.14). Taxi drivers had higher odds of testing positive (OR 4.08, p<0.001) than non-drivers while holding constant other characteristics at their respective base values. In sensitivity analyses, where results were restricted to males in the study population, we identified similar trends for age, occupation and region (results not shown).

Prolonged positivity

The graphical results from the TTE analysis considering testing 'negative' as an event after testing positive for COVID-19 are presented in Figure 4. The overall mean and median positivity periods were 17 and 15 d, respectively. However, the disaggregated analysis over sex, age and pre-symptomatic nature revealed
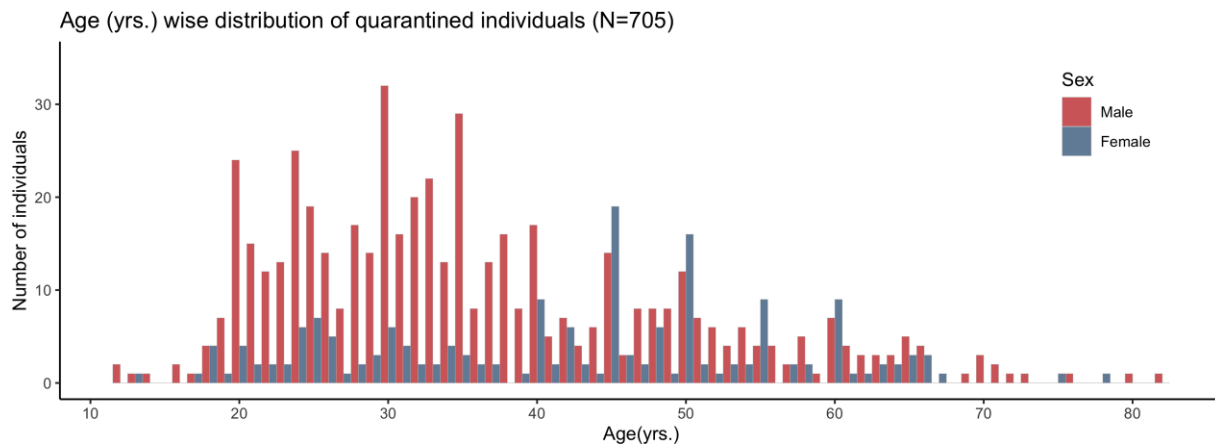
Figure 2. Age (years) and sex distribution of quarantined individuals (N=705).
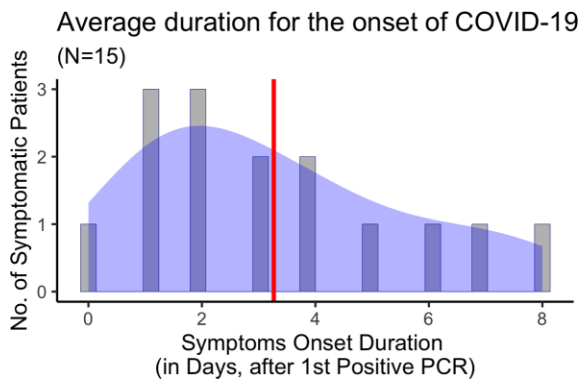

Figure 3. Average onset of COVID-19 symptoms after first positive PCR test (days after first positive PCR) (n=15).

the differences in the prolonged positivity period for our study cohort. For example, even though statistically non-significant, the median positivity days were 11 for females vs 14 for males (χ^2=2.6, p=0.1). The age-group wise Kaplan–Meier curve showed that the individuals 21–40 y of age had more cases that could

go beyond the 14-d positivity period, however, the positivity time period did not differ significantly across all age groups.

The most important results were obtained for the symptomatic (median positivity time 26 d) and asymptomatic (median positivity time 14 d) group comparison (χ^2=11.4, p<0.001) (Figure 4a). Further, as shown in Figure 4b, the risk of testing negative at any given time during the quarantine was 0.32 times (p<0.01) lower among the pre-symptomatic patients when compared with the asymptomatic individuals after adjusting for sex, age, occupation and residence. In other words, in our study cohort, symptomatic individuals had a greater risk of staying COVID-19 positive for a longer period than the asymptomatic patients after controlling for other factors.

Discussion

In this period prevalence study we found evidence of a substantial infection level with SARS-CoV-2 in asymptomatic travellers. Infection was common in younger men, partly attributable to the exposure risk while working in communities as taxi or truck drivers and coming in contact with infected individuals. Another study[16]

Table 2. Asymptomatic and pre-symptomatic distribution across gender and age groups. The observed distribution for asymptomatic and pre-symptomatic cases did not differ across sex and age groups (i.e. Fisher's exact test was statistically non-significant at p=0.9 and 0.05, respectively)

Variable	N	Asymptomatic (n=56)	Pre-symptomatic (n=15)	p-Value[a]
Sex, n (%)	71			>0.9
Male		51 (91.1)	14 (93.3)	
Female		5 (8.9)	1 (6.7)	
Age group (years), n (%)	71			0.050
≤20		10 (17.9)	0 (0)	
21–40		39 (69.6)	10 (66.7)	
41–60		6 (10.7)	3 (20.0)	
≥61		1 (1.8)	2 (13.3)	

[a]Fisher's exact test.

Table 3. Odds of testing positive

Variable	OR	95% CI	p-Value
Driver			
Non-driver	–	–	
Driver	4.08	2.30 to 7.37	<0.001
Sex			
Male	–	–	
Female	0.49	0.18 to 1.18	0.14
Age group (years)			
≤20	–	–	
21–40	0.49	0.22 to 1.13	0.079
41–60	0.26	0.09 to 0.71	0.008
≥61	0.44	0.09 to 1.64	0.3
Residence			
Peshawar	–	–	
Khyber	0.73	0.40 to 1.31	0.3
Other	0.52	0.23 to 1.09	0.093

CI: confidence interval.

noted that work-related transmission other than in healthcare workers plays an important part in transmission, as in our study, where prevalence is high in taxi and truck drivers frequently crossing the border. The third most affected population is housewives, who do not have any direct exposure to the virus. They might have been exposed to the virus from their working counterparts. Similar findings of spread of the virus to households by working individuals is described by other studies[17] as well.

Our study captures the differential risk of transmission in pre-symptomatic and asymptomatic individuals. As noted by He et al.,[18] identifying this difference is critical in understanding the community transmission and effective contact tracing of COVID-19. We further track the duration of onset of symptoms and show that the positive patients in our sample developed symptoms on average 3.3 d after PCR testing. This interval between contracting

the coronavirus and displaying symptoms is critical in transmitting the infection to healthy individuals, as shown by Wei et al.[19] The proportion of asymptomatic individuals is higher in our sample (85% of positive cases). Symptomatic patients may exhibit protective behaviour by physical distancing, hand washing, wearing face masks and avoiding crowded places. In contrast, the asymptomatic young individuals will carry on everyday activities and act as vehicles for viral circulation in their communities unless detected in time.[23,25] Educational institutions and businesses are now open and restrictions have been lifted for the general society.[24] Pakistan has a large younger demographic that increases the importance of early detection of positive cases for effective isolation. Moreover, the vaccination coverage is higher in older individuals and the younger population poses the risk of driving outbreaks in the community.

Furthermore, we also show that individuals <20 y of age have a higher prevalence of asymptomatic cases. A total of 14% of all our positive cases were positive in this age group, but none developed any symptoms. There are two possible explanations for having a high proportion of asymptomatic cases in younger individuals. First, we excluded individuals who were symptomatic at the time of screening and were directly sent to the health facility. Therefore we could not capture the full spectrum of disease spread in this specific age group of travellers. Second, the literature suggests that the clinical representation of disease may vary across ages.[21,22] Our results from Table 2 hint towards this possibility, as older individuals have developed symptoms in our sample. In this context, it is important to note that the younger cases could be a potential infection source to their families if they are not isolated. Our finding is important evidence for extending the testing and isolation of younger individuals during contact tracing.

Our TTE analysis shows that symptomatic patients had a longer positivity period in our cohort. It re-emphasizes the need for effective isolation of symptomatic cases until they fully recover from the infection. However, even with a shorter positivity period, tracking and isolating of asymptomatic patients is important, as studies[20,25] indicate that the majority of transmission occurs from people who do not exhibit any symptoms.
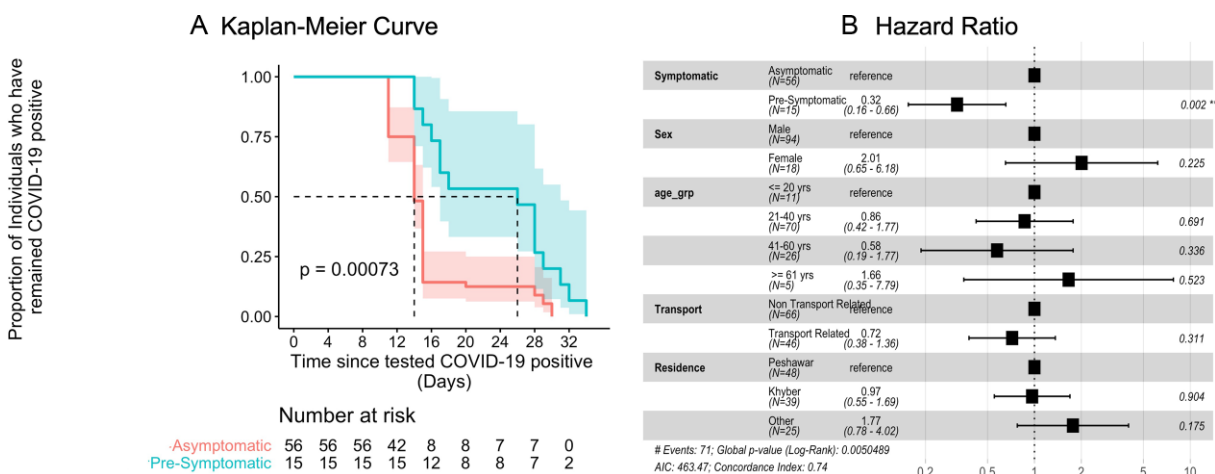

Figure 4. TTE analysis and hazard ratios. **(A)** Kaplan–Meier curve shows the risk of staying positive was higher among pre-symptomatic patients. **(B)** Hazard ratios. The risk of testing negative at any given time during the quarantine was 0.32 times lower (p<0.01) among the pre-symptomatic patients compared with the asymptomatic individuals.

Another important aspect of asymptomatic viral circulation is the critical role that it can play in the emergence of new genetic variants.[26] This link needs to be studied further.

There are a few limitations to our study. First, a single negative PCR test cannot confidently rule out an asymptomatic person with the disease. A repeat test is usually required, which was not done in our study. We might have missed a few cases and hence underestimated the period prevalence. Second, due to government rules and regulations, we could not access the individual cycle threshold values for each test, as it was only reported as a qualitative test and not a quantitative test. Having access to these data would have shed more light on viral load in each positive patient. Lastly, no laboratory was studying the genetic sequencing of the virus at the time of the study in Khyber Pakhtunkhwa province and only a single lab was designated to test for SARS-CoV-2 virus. Therefore we were unable to state which virus strain was circulating in our study population.

Conclusions

In this study we determined that 10% of the border quarantine residents tested positive and the majority of them remained asymptomatic. We further show that young males in certain risky occupations, like taxi and truck driving, are more prone to acquiring COVID-19 infection. Moreover, we demonstrated the critical time of symptom onset among the pre-symptomatic patients. This can help in timely isolation of such cases. It is recommended that testing capacities should be enhanced and asymptomatic individuals involved in risky professions should be screened and tested in time to stop subclinical spread of the disease and the emergence of new variants.

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