From Infection to Immunity - Impact of COVID-19 Across Nine Hemodialysis Centres in Mumbai

Abstract
Introduction: There are several studies of symptomatic hemodialysis patients with proven COVID-19 infection. However, there is paucity of data on asymptomatic COVID-19 infection in the outpatient hemodialysis population. The true prevalence and transmission of this infection in hemodialysis centres is unknown. This study was conducted across hemodialysis centers by testing all patients and staff for COVID-19 PCR and later for IgG antibody, irrespective of their symptoms. Methods: All 705 hemodialysis patients and 103 dialysis staff across nine centres, were tested for COVID-19 over a period of 54 days of the pandemic, and for COVID IgG antibody of available enrolled staff and patients, after 8 weeks of study termination. Results: The period prevalence of infection in patients and staff was 7.1% and 14.6% respectively. Mortality in patients was 18%, and all staff recovered. Clustering of patients and staff occurred at 3 of 9 centers. Of 26 HIV positive patients, only one contracted the COVID-19 infection and has recovered. Of those infected, seroconversion occurred in 80% of patients and 83% of staff. Seroconversion also occurred in 16% of patients and 37% of staff, who were asymptomatic and COVID PCR negative during the study period. Conclusions: Testing a patient only when symptomatic, identified only 26% (13/50) of infected patients. For every single symptomatic patient who tested positive, there were 3 other asymptomatic infected ones. There was a high seroconversion rates in infected subjects. But antibodies also developed in asymptomatic subjects, indicating silent transmission and antibody generation in this population.

Keywords: Clustering, COVID-19, hemodialysis, HIV, seroconversion, staff

Introduction
Outcome of SARS-CoV-2 infection in the general population and organ transplant recipients have been reported recently. However, the true burden and outcome of COVID-19 infection among hemodialysis patients and staff at hemodialysis centres have not been established. It is plausible that the spread of COVID-19 infection may occur within a hemodialysis centre from patient to patient as well as to staff. Hence we conducted a study wherein we tested all patients and all staff within these dialysis units, during the pandemic and evaluated the burden and outcome of COVID-19 infection among hemodialysis patients as well as dialysis staff, irrespective of their symptoms.

Study Methods
This was a prospective observational study conducted at 9 hemodialysis centres in Mumbai, between March 24 and May 17, 2020. Institutional Review Board permission was obtained for this study. All hemodialysis patients (n = 705) and staff (n = 103) caring for patients at these nine centres were included in this study. There were no exclusion criteria. All staff were provided with N 95 masks and PPE equipment, and were trained to use them. Every patient and staff member was tested for COVID-19, either because of COVID-19 symptoms or as a screening protocol. This was done as a research protocol only. A nasopharyngeal swab was used for detection of 2019-nCoV RNA which targets the B- CoV (target E gene) and E SARS-CoV-2 (S gene) by a real-time qualitative RT-PCR method. Patients and staff who tested positive were either admitted into a hospital or a designated quarantine facility based on the severity of their illness and treated as per prevailing protocols. The demographic data of patients and staff as well as comorbidities in the patients including Diabetes (DM), Hypertension (HTN), Ischemic heart disease (IHD), Tuberculosis (TB), lung
disease, Peripheral vascular disease (PVD) and HIV infection were recorded. All available infected and non-infected, patients and staff were tested for COVID-19 antibodies, after 8 weeks of study conclusion. The Euroimmun AG IgG ELISA assay against the viral S1 region of the spike protein was used to quantify IgG antibodies in serum.

We used R Programming language for all statistical analysis. T-test, KS-test, Chi-square tests were used for statistical analysis. Descriptive statistics were reported using mean, median and standard deviation (SD) for continuous variables and percentage and confusion matrices for categorical variables. \( P < 0.05 \) was considered as significant.

**Results**

We tested 808 cases (705 patients and 103 staff) for COVID-19 infection. The total number of patients and staff who were tested, number of patients who were positive for COVID-19 and their outcome are shown in Figure 1.

**COVID-19 infection in patients on MHD**

Fifty (7.1%) out of 705 patients on MHD were found to be infected with COVID-19. Key demographic variables of staff and patients with and without COVID-19 infection are shown in Table 1. MHD patients with COVID-19 infection were younger than those without infection (\( P = 0.04 \)). Symptoms suggestive of infection prompted testing in 13/50 (26%) patients. Fever was the commonest symptom (100%), followed by fatigue (46%), cough and dyspnoea (38%) and diarrhoea (15%). The remaining 37/50 (74%) were asymptomatic and COVID-19 infection was detected through routine screening. Hypertension was the commonest comorbidity (88%), followed by Diabetes (34%), IHD (14%) and Tuberculosis (8%).

**Outcome of COVID-19 infection in MHD patients**

Among 50 patients with infection, 42 patients (84%) were hospitalised, 6 patients (12%) were admitted to a quarantine facility and 2 patients died at home before hospitalisation. The mean duration of hospitalisation was 11 ± 5 days. Death occurred in 9/50 (18%) patients of which 2 died within 24 h of diagnosis of COVID-19 infection. The remaining 7 patients died of COVID-related severe acute respiratory illness. Symptoms at diagnosis were noted in 7/9 patients who died. Older age (\( P = 0.018 \)) and Diabetes (\( P = 0.029 \)) were significant risk factors identified for death associated with COVID-19 infection [Table 2]. At the time of this report, all the surviving patients were discharged from the hospital.

**COVID-19 infection in HIV positive MHD patients**

A total of 26/705 patients on MHD had concomitant HIV infection. Only 1/26 (4%) of them had COVID-19 infection and was asymptomatic. Of this group, 22 patients were on triple therapy and 4 were on dual drugs. Only 20% patients were on a combination which included the protease inhibitors, Lopinavir, Ritonavir and Atazanavir. The rest were on combination therapy with Lamivudine, Abacavir, Nevirapine or Efavirenz. Of the 26 patients, 19 had undetectable HIV viral loads with a median CD4 count of 545 (range 237-1020). The remaining 7 had a median viral load of 20 with a CD4 count of...
Billa, et al.: COVID-19 infection in hemodialysis centres

531 (range 134-1050). The single asymptomatic HIV patient who tested positive for COVID-19 also had a coinfection with Hepatitis B and C. His viral load was 20 and CD4 count was 510. He was quarantined and recovered [Table 3].

COVID-19 infection in dialysis staff

COVID-19 infection was noted among 15/103 staff, making the overall period prevalence of infection among hemodialysis staff to be 14.5%. This was double the prevalence of COVID-19 infection in MHD patients. All these staff were residing on the premises of the dialysis center itself. Key demographic variables in staff with and without COVID-19 infection are shown in Table 1. The mean age of dialysis staff (28 ± 8 years) was significantly lower than dialysis patients (55 ± 14 years) (P < 0.001). All infected staff were quarantined and had an uncomplicated course with complete recovery.

COVID-19 infection within dialysis centres

Three out of 9 hemodialysis centers noted 31/50 (62%) patients with COVID-19 infection. Eleven out of 15 (73%) staff with the infection were also clustered in the same 3 centers. In addition, 50% of infected patients at one of these centres were undergoing dialysis concurrently in the same shift. Thus, It is conceivable that there was exposure and infection spread between patients and staff. A similar exposure between staff could be contributed by their residing on the same premises during this period.

Viral clearance of COVID-19 infected MHD patients

Nine patients were tested for the virus only once, either because they died (n = 6) or because they were in a quarantine facility where retesting was not mandatory (n = 3). The remaining 41 were retested more than once (3.1 ± 1.3). The follow-up test was negative in 30/41 (73%) after a median period of 12 days. Conversion from positive to negative occurred in 52% by day 7, 78% by day 12, 92% by day 14, and 96% by day 17.

Results of Antibody Testing

All available COVID-19 infected and non-infected patients and staff at the 9 dialysis centres, were tested for IgG antibodies to SARS-CoV-2, after 8 weeks of the viral testing. Out of the 50 COVID-19 PCR positive patients, 41 survived. Of this 36 patients were available for IgG antibody testing (Positive titre >1.1). Antibody positivity was found in 29/36 patients (80%) with a mean titre of 4.17 ± 2.15. Out of the original 655 COVID-19 negative patients in the study, 74 patients were tested for COVID antibody, of whom 12 patients tested positive (16%), with a mean titre of 2.19 ± 1.03. None of these 74 patients had symptoms suggestive of COVID during the interval period.

Of the 103 staff members who were included in the study, 85 were available for antibody testing. Twelve of them
were originally PCR positive, of whom 10 were detected to be antibody positive (83%), with a mean 3.85 ± 2.43. The remaining 73 staff were originally PCR negative, of whom 27 were detected to be antibody positive (37%), with a mean titre of 3.68 ± 1.62 [Table 4].

Discussion
The COVID-19 infection has so far affected 215 countries around the world. As of July 5, 2020 there have been 11,769,319 cases across the world of which India ranks third and harbours 7,22,007 cases. The worldwide mortality in the infected population stands at 5,41,488 cases and the mortality figures for India stand at 20,185 cases. The city of Mumbai had so far recorded 85,724 cases with approximately 1000 new cases being detected each day with an average mortality of 70 cases each day.[5]

Incidence of COVID-19 in MHD patients
There is wide variability in the reported prevalence of COVID-19 infection in MHD patients. In the Wuhan study, 154 symptomatic patients at 65 dialysis centres (total patients 7154) had confirmed COVID-19 infection (2.15% in symptomatic MHD patients).[3] Similarly in Lombardy, the prevalence of this infection in the symptomatic hemodialysis patients was reported as 5% with a mortality of 22.3%.[6] The Spanish COVID-19 registry documented 547 symptomatic patients on MHD with COVID-19 infection.[7] The above studies only evaluated symptomatic patients therefore perhaps underestimating the actual infected population. The prevalence of COVID-19 infection in the entire hemodialysis population in our study was 7.1%. Symptomatic patients formed 1.6% and asymptomatic patients formed 5.5% of this population.

Recent studies suggest that 86% infections can remain asymptomatic and therefore undocumented. The transmission probability from them appears to less than half that from symptomatic positive cases.[8] The Brescia Renal Covid Task Force studied the short term outcome of MHD patients with COVID-19 infection, in 4 hemodialysis centres. The positivity rates were 14% (symptomatic patients at 3 centres) and 16% (all patients at 4th centre) concluding thereby that there is no substantial difference in the two approaches.[9] In contrast, our study demonstrates a significant burden of asymptomatic COVID-19 positive patients (74%) in dialysis centres. In one study, asymptomatic infection was associated with a higher likelihood of nasopharyngeal viral RNA clearance within the first week of diagnosis compared with symptomatic infection.[10] It is yet unclear whether the proportion of symptomatic and asymptomatic patients is related to an impaired immune response due to underlying CKD.[11]

Clustering of COVID-19 cases
Dialysis patients and staff are forced to share a common area, thus predisposing to transmission. As in the Lombardy study, clustering of infected cases was seen in 3 of 9 centres in our study, indicating cross infection between patients and staff.[6] This effect could also have been contributed by the staff residing on the same premises during the lockdown period as well as seen in dialysis units during the MERS epidemic in Saudi Arabia.[12]

Risk factors for mortality in ESRD
Older age, cardiovascular disease, diabetes, chronic respiratory disease, hypertension, and cancer were all associated with an increased risk of death in COVID-19 infection.[13] Patients on MHD are high risk as they have the same comorbidities.[14] The mortality rates due to COVID-19 was 29% in Brescia, 30.5% in Spain and 16.2% in Wuhan.[8,15,16] Our study reports a mortality of 18%, similar to that in Wuhan.

COVID-19 infection in HIV positive patients on hemodialysis
There is limited data available on the prevalence of COVID-19 in HIV positive hemodialysis patients. HIV patients accounted for 1% of all COVID-19 hospitalisations in Spain. There were no MHD patients in this group.[17] Only a single HIV positive MHD patient out of 26, tested positive in our study, although this low prevalence was not statistically significant.

COVID-19 infection in hemodialysis staff
The Wuhan study had a lower rate of infection in hemodialysis staff (2.9%) as compared to our study (14.5%).[18] This higher infection rates in staff in our study is likely due to the comprehensive screening protocol adopted as well as the longer duration of the study period (8 weeks) as compared to the Wuhan study (2 weeks). Many of these staff in our study, were residing on the premises of the dialysis units due to the logistical challenges. The fact that they were significantly younger than the patient population ($P < 0.001$) and

<table>
<thead>
<tr>
<th>Table 4: Seroconversion rates in Hemodialysis Patients and Staff</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Status</strong></td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Patients</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Staff</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

[Downloaded free from http://www.indianjnephrol.org on Wednesday, October 6, 2021, IP: 125.99.35.202]
lacked comorbidities could potentially have influenced this positive outcome.

Viral clearance

How long a person remains infectious after acquiring the COVID-19 infection is uncertain. Our study reported a 96% clearance rates by day 17, whereas data from the UK which reported the results of retesting in 34 MHD patients after COVID-19 diagnosis; only 15% patients cleared the virus in less than 11 days, and 59% by day 15.[19] The available data suggest that prolonged viral RNA shedding after symptom resolution is not clearly associated with prolonged infectiousness.[20-22]

Antibody testing

COVID-19 IgG antibody titres increase over time, with better clinical sensitivity demonstrated between samples that were taken >14 days compared to <14 days after the onset of clinical illness. This finding underscores the important point that sampling after 14 days may thus serve as an adequate threshold for testing to optimize retrospective identification of COVID-19 infected patients.[23] In our study, 80% of the COVID PCR + patients, and 83% of COVID PCR + staff, were found to have antibodies when we tested for them after a minimum of 8 weeks after positivity. This is consistent with previous studies which reported a seroconversion rate of 64.7% to 100% for IgG after an interval ≥2 weeks.[24,25] Some patients and staff developed IgG antibodies despite testing negative for COVID-19 PCR and remaining asymptomatic on followup. This occurred in 16% of patients and 37% of staff, indicating asymptomatic transmission of the infection and antibody generation in this population. In these subjects, IgG antibody titres were lower than those who had documented infection. The role of seroconversion on development of long term immunity, is the subject of ongoing research.[26]

There are some limitations to our study. Each of these patients who tested negative during a one time testing, were potentially at risk for getting infected on any day after this primary testing. If symptomatic, there would have been grounds for retesting them. If asymptomatic, it would be very difficult to keep testing them serially and repeatedly to identify new infections especially in asymptomatic patients. Hence the antibody testing was done. This helped to identify those initially negative patients who could have contracted an asymptomatic infection during the period after primary PCR testing. Thus antigen and antibody results, taken together, convey the true burden of the infection in dialysis patients.

Conclusion

In summary, this study identifies the significant burden of COVID-19 infection in outpatient hemodialysis patients as well as staff. It clearly shows clustering of cases in dialysis units, indicating transmission of this infection between patients and staff. This underlines the need for adopting a low threshold for testing as well as isolating the infected cases. Since asymptomatic cases formed the vast majority, its mandatory to follow strict personal protection protocols by staff. Dialysis patients by virtue of their comorbidities are at higher risk for mortality. Although there was a high prevalence of infection in hemodialysis staff, they had an uneventful course because they were younger and lacked comorbidities. Antibody testing helps in identifying COVID-19 infected patients, retrospectively.

Acknowledgements

The authors thank the services of Salman Arif who did the statistical analysis for this project.

Financial support and sponsorship

The authors thank the Apex Kidney Foundation for an academic grant to cover the cost of all PCR testing as well as the antibody testing for this study.

Conflicts of interest

There are no conflicts of interest.

Bibliography


