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Rate of shed of SARS COV-2 viral RNA from COVID-19 cadavers

Meenakshi Sharma, Megha Brijwali, Nababarun Chakraborty, Aashish Choudhary, Arbind Kumar, Sharad Srivastav, Parin Lalwani, Richa Agrawal, Kapil Dev Soni, Nirupam Madaan, Rajesh Malhotra, Purva Mathur, Sanjeev Lalwani, Lalit Dar, Anjan Trikha

Background: At what rate does the RNA of SARS CoV-2 shed from cadavers? Although, there have been numerous studies which have demonstrated the persistence of the virus on dead bodies, there is a lack of conclusive evidence regarding the variation of viral RNA content in cadavers. This has led to a knowledge gap regarding the safe handling/management of COVID-19 decedents, posing a barrier in forensic investigations.

Methods: In this study, we report the presence of RNA of SARS CoV-2 by real time RT-PCR, in nasopharyngeal swabs collected after death from two groups of bodies – one who died due to COVID-19 and the other who died due to other diagnoses. A prospective study on 199 corpses, who had tested positive for COVID-19 ante-mortem, was conducted at a tertiary care center. RNA testing was conducted at different time intervals (T1-T5).

Results: 112 (56.3%) died primarily due to COVID-19 and 87 (43.7%) died due to other diagnoses. 144 (72.4%) were male and 55 (27.6%) were female. A total of 115 (57.8%) tested positive for COVID-19 after death at different time points. The mean age was 50.7 ± 18.9 years and the length of hospitalization ranged from 1 to 50 days with a mean of 9.2 ± 7.6 days. Realtime RT-PCR positivity of SARS CoV-2 RNA decreases with time.

Conclusion: We observed that real time RT-PCR positivity, indicating viral RNA detection, decreases with time. Therefore, it is advisable to follow appropriate COVID-19 precautions to carry out scientific studies, medico-legal investigations and mortuary services on suspected/confirmed COVID-19 corpses.

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Introduction

The SARS-CoV-2 virus is ravaging the world with newer variants and higher transmission potentials, with over six million deaths worldwide. First identified in China, the World Health Organization declared the outbreak a Public Health Emergency of International Concern (PHEIC) on 30th January, 2020 and a pandemic on 11th March same year [1]. With global tallies rising exponentially, the pandemic has created fear, anxiety and panic about the preparation and burial of bodies with suspected/confirmed COVID-19. Cultural and religious traditions too dictate how a body is to be handled and these vary across different ethno-religious groups and geographical terrains. Persistence, transmissibility and disease morbidity have been variable, not only across different strains, but also across different countries for the same strain with probable influence from host genetics and environmental influences.

The virus primarily spreads through aerosolized droplets of infected persons but transmission via COVID-19-infected decedents remains uncertain [2], which necessitates research in this area. A 2005 study from China reported that there was no transmission of SARS CoV-2 from the deceased to healthcare professionals in the BSL-3 autopsy area [3]. However, Liu et al. reported that approximately 28% healthcare workers in contact with deceased COVID-19 bodies contracted the virus [4]. A short communication from Thailand described a forensic practitioner who had contracted the virus after being in contact with biological samples, although, this was a mere possibility [5,6]. In another media report in 2020, a funeral home worker claimed to have been infected, following transport and preparation of a COVID-19 cadaver, without any other firmly established transmission routes [7].

Persistence of viral RNA in post mortem settings for many viruses, let alone SARS CoV-2, remains unexplored despite myriad risks associated with transmission. Nipah, a zoonotic virus that transmits via respiratory droplets, has reportedly exhibited corpse-to-human transmission due to improper handling [8]. Lungs of patients with influenza, if handled carelessly during an autopsy, can be infectious as well [9,10,11] Transmission can happen due to accidents with sharp contaminated objects, such as needles, scalpels etc. or accidents involving skin lesions during surgical/autopsy procedures. While the duration of viral shedding is unique to each virus, COVID-19 is known to range at a median of 14 days with an interquartile range of 12 days in survivors.

Evidence regarding transmission of the SARS CoV-2 virus from the deceased to the living remains contradictory and inconclusive. Hence, there is a need for persistence studies in human remains to support proposed management strategies that are efficacious, practical, economically feasible and culturally acceptable.

Risk of infection decreases with time [12], but the real question is, at what rate? Without a fair idea of the potential risks dead bodies pose, limited numbers of autopsies have been performed on bodies with suspected/confirmed COVID-19 cases throughout the world. In fact, due to asymptomatic carriers, the risk is present in virtually any cadaver that autopsy and funeral workers have to deal with [13].

Utility of proper diagnosis in forensic investigation is imperative, as for medico-legal cases the situation gets all the more complex. Workers may be confronted with legal mandates to perform autopsies and other forensic pathological studies on violent deaths under suspicious circumstances. However, some jurisdictions prohibit invasive procedures such as autopsies on COVID-19 positive bodies, despite the medico-legal repercussions and research benefits [14,15]. Autopsies may also be necessary to determine if a death is due to COVID-19 or not, as discrepancies between clinically determined cause of death and autopsy observations are well documented, and have significant impact on statistical data [16]. Autopsies also help in examining inaccessible body tissues, identifying pathophysiology and mechanisms of action that could eventually lead to innovative therapeutic approaches. Thus, there is an urgent need to optimize postmortem diagnostic techniques. Healthcare staff including forensic experts ought to be aware of the persistence of SARS CoV-2 RNA in cadavers and the time period until testing may help in diagnosis and ascertain cause of death as well.

In this study, we detect the presence of viral RNA in nasopharyngeal swabs of deceased COVID-19 positive patients at different time intervals. The viral detection was further analysed with clinical and epidemiological parameters. We attempted to determine the time for which suspected or confirmed COVID-19 decedents no longer harbor the viral RNA and may safely be handled. This may enable autopsies to be performed and thus aid in the proper determination of cause of death, particularly in the investigation of COVID-19 deaths. Our study happens to be the first with a diverse cohort and significant observation time after death.

Methodology

Case selection

This prospective study was carried out at JPN Apex Trauma Centre, AIIMS, New Delhi, India, that was a dedicated COVID Care Centre, during the COVID-19 pandemic. In the study period of April 2020 to March 2021 a total of 1479 deaths were recorded at our hospital.

Inclusion criteria

Only cases with complete clinical history were enrolled in the study. This included in-hospital patients who died with positive RT PCR report for COVID-19 and brought-dead cases who tested RT PCR positive for COVID-19 in the emergency department.

Exclusion criteria

There were no clinical or epidemiological restrictions on inclusion of cases. However, we excluded cases where consent from legally authorized representative (LAR) was not obtained. We also avoided sample collection from unclaimed/unknown bodies in view of ethical mandates and non-availability of clinical documentation.

A hundred and ninety-nine deceased were included in this study. The subjects were selected randomly and the groups were similar with respect to age and sex, as well as in presentation of clinical symptoms and comorbidities. (Table 1). This included those who had tested positive for COVID-19 at admission (139), or incidentally discovered COVID-19 infection during hospital stay [25], or brought dead (35). Their ante-mortem reports for SARS-CoV-2 RNA was positive by RT-PCR, CBNAAT, RAI or a combination of these. All bodies in the “brought-dead” group were screened at the hospital emergency on arrival and were included only if they tested positive by RT-PCR and with due availability of ante-mortem clinical reports. Furthermore, based on Medical Certification of Cause of Death (MCCD) and clinical histories, the cohort was divided into two subgroups – a) patients who died primarily due to COVID-19 (n = 112); b) patients who died due to other diagnoses but had positive RT PCR report for COVID-19 (n = 87) (Fig. 1).
Sample collection

All deceased bodies were shifted to the mortuary and preserved at a temperature of approximately 4 °C. Nasopharyngeal swabs were collected from each of these bodies. To maintain homogeneity in the sampling and testing procedures, a single operator was designated for sample collection and all samples were tested at a single site, the virology laboratory. We collected samples over a significant amount of time after death (0hrs to > 120hrs).

Nasopharyngeal swabs were collected as per the U.S. Centre for Disease Control (CDC) interim protocol for collecting, handling, and testing clinical specimens from the deceased. Samples were collected at varying time periods and for ease of analysis five time slots were demarcated corresponding to different time intervals after death (T1: 0–24hrs, T2 > 24–48hrs, T3 > 48–72hrs, T4 > 72–96 hrs, T5 > 96–120 hrs).

| Time Groups | Age [Mean ± SD] | LOS [Mean ± SD] | Ct [Mean] | Died due to COVID-19 (Total N) | Died due to other diagnoses (Total N) |
|-------------|----------------|----------------|---------|-------------------------------|-------------------------------------|
|             |                |                |         | Positive [n(%)]               | Positive [n(%)]                     |
| 0–24 hrs    | 51.6 ± 18.1    | 8.5 ± 7.2      | 26.9    | 89                            | 64 (72)                            |
| 24–48 hrs   | 54.4 ± 19.4    | 9.5 ± 7.2      | 25.6    | 13                            | 10 (77)                            |
| 48–72 hrs   | 52.2 ± 19.8    | 9.7 ± 9        | 27.5    | 2                             | 1 (50)                             |
| 72–96 hrs   | 49.5 ± 16.4    | 13 ± 6.8       | 28.1    | 5                             | 3 (60)                             |
| 96–120 hrs  | 50.0 ± 16.5    | 10.7 ± 11.9    | 29.6    | 3                             | 1 (33.3)                           |

Note: SD = Standard Deviation, Ct = Cycle threshold, E gene = Envelope small membrane protein gene. RT-PCR = Real-time reverse transcriptase-polymerase chain reaction.

Table 1
Epidemiological profile.

Fig. 1. Schematic Diagram of study: This diagram depicts the selection of cases and division of subjects in different time slots (T1-T5). Note*: LAR: Legally authorized representative T1: 0–24h, T2 > 24–48h, T3 > 48–72h, T4 > 72–96 h, T5 > 96–120 h.
T5 > 96–120hrs. Sequential sampling on the same corpse was not possible due to ethical reasons as we could not keep the same bodies for the entire duration of the study. The bodies had to be dispatched for cremation as per the wishes of the family or/and other clearances as necessary. Tests were done as per availability of resources and manpower during the pandemic surge.

Data collection

All documents available at the center concerning the deaths were evaluated descriptively. Cause of death statements were documented in accordance with the guidelines of the World Health Organization [17].

PCR testing

Samples received at the virology laboratory were processed and centrifuged at 1500 g for 15 min. Supernatant was aliquoted in cryovials and stored at −80 °C. RNA was extracted with the MagMAX automated extraction system using MagMAX Viral/Pathogen II Nucleic Acid Isolation kit (Thermo Fisher Scientific Inc., US) following the 200 µL sample input volume protocol and eluted in 50 µL volume, as per the manufacturer’s instructions.

Real time RT-PCR testing for SARS-CoV-2 was performed by Indian Council of Medical Research (ICMR) approved testing kits. Over the period of study, as per availability, Indian testing kits from ICMR-NIV, TRUPCR, and COVIDsure were used. Tests were performed on the Agilent AriaMxReal-Time PCR system (Agilent Technologies Inc., USA). A human housekeeping gene RNaseP or B-actin was also simultaneously detected as internal control in order to assay sample quality.

The ICMR-NIV Real Time RT-PCR assay version 3.1 (ICMR-National Institute of Virology, Pune, India) included three targets- E gene, ORF1ab gene and RdRp gene of SARS-CoV-2 genome with B-actin as the internal control. The TRUPCR SARS-CoV-2 RT qPCR kit version 2.0 (Improved) Real Time RT-PCR kit (Kilpest India Ltd., Bhopal, India) targeted E gene and N gene of SARS-CoV-2 genome, while the COVIDsure Multiplex Real Time RT-PCR kit (Trivitron Healthcare Pvt. Ltd., Chennai, India) used E gene and ORF1ab gene targets. The human RNaseP P gene served as an internal control in the latter two kits. Results were analyzed as per the manufacturers’ instructions.

A consensus cycle threshold (Ct) cut-off of ≤ 35, with a sigmoid curve was considered positive for qualitative interpretation, as per the ICMR advisory. The Ct value measurement for the E gene target was used as a surrogate marker for semi-quantification of viral load. The Ct value is defined as the amplification cycle number at which the fluorescent signal crosses the threshold. Therefore, the amount of SARS-CoV-2 RNA present in the patient sample is inversely related to the Ct value. Ct values thus obtained were grouped into three categories as follows: ≤25: strongly positive; 25–30: moderately positive; and > 30: weakly positive for both groups.

Statistical analysis

Summary statistics were reported as means with standard deviations for continuous variables, and frequency with proportions for categorical variables. For both groups, enumerated as died due to COVID-19 and died due to other diagnoses, the percentage of positive PCR results was plotted against 24-hour time intervals to reveal the trends and extrapolate the graph to obtain the time at which the results turn potentially negative. Bar plots were used for visualizing viral load in corpses that died due to COVID-19 vs. patients that died due to other likely diagnoses. STATA version 11.1 (Stata Corp., College Station, TX, USA) was used for statistical analyses.

Ethics approval

Sample collection was performed only after taking informed consent from the LAR of the deceased, in accordance with the Declaration of Helsinki [18].

Results

Demographic data

A hundred and ninety-nine patients who had tested positive for COVID-19 were included in this study. This included patients who had tested positive for COVID-19 at admission (139), or incidentally during their hospital stay (25), or brought dead (35). All in-hospital patients or brought dead bodies included in the study had documented COVID-19 positive test result (ante-mortem or immediately after death in hospital emergency). Based on clinical history and MCCD, it was noted that 112(56.3%) died primarily due to COVID-19 and 87(43.7%) died due to other diagnoses.

Out of these 144(72.4%) were male and 55(27.6%) were female. A total of 115 (57.8%) tested positive for COVID-19 after death at different time points. The mean age was 50.7 ± 18.9 years and the length of hospitalization, i.e., the time interval from admission to death, ranged from 1 to 50 days with a mean of 9.2 ± 7.6 days (Table 1). These decedents came from different parts of India, with ample regional-cultural diversity and hence, the forthcoming results may safely be generalized to the South Asian context.

Persistence studies

The six groups were designated on the basis of time interval and revealed the corpses to be weakly to strongly positive, albeit in varying degrees. Nasopharyngeal swabs were collected within 0–24 h of death (T1), 24–48 h (T2), 48–72 h (T3), 72–96 h (T4) and 96–120 h after death (T5). The number of subjects in each of these groups was: n1= 139, n2= 27, n3= 12, n4= 6, n5= 15 with Ti corresponding to ni, i being the ith variable.

RT PCR test

Our cohort comprised of two subgroups - one who died primarily due to COVID-19 and the other who died with COVID-19 albeit due to other likely diagnoses. In both these groups, the percentage of corpses that gave a positive RT PCR result post mortem decreased with time. For the first sub-group i.e. cases that died due to COVID-19, the percentages are as follows: 72%(T1:0–24hrs), 77%(T2: > 24–48hrs), 50%(T3: > 48–72hrs), 60%(T4: > 72–96hrs) and 33.3%(T5: > 96–120hrs) with p value > 0.05. The second sub group i.e patients who died due to other diagnoses, revealed the following: 42%(T1:0–24hrs), 71%(T2: > 24–48hrs), 30%(T3: > 48–72hrs), 0%(T4: > 72–96hrs) and 17%(T5: > 96–120hrs) with p-value 0.05 (Fig. 2).

Ct value

We also observed that Ct values related well with the cause of death. Among those who died primarily due to COVID-19, 76% corpses had high viral load. At a stark contrast only 44% of subjects who had died due to other diagnoses exhibited similar profiles (Fig. 3).

Clinico-epidemiological profile

Most patients were found to have associated underlying illnesses. In descending order of prevalence, hypertension (56%), diabetes mellitus (53%) and cardiovascular problems (25%) were found to be the major associated diseases, followed by cancer (23%), chronic kidney disease (20%), chronic liver disease (16%) and bronchial...
asthma/TB (8%). Other associated comorbidities were hypothyroidism (3.5%), obesity (2%), bacterial meningitis (0.5%), cholelithiasis and pancreatitis (1.5%), obstructive sleep apnea, mucormycosis (1.5%), Down’s syndrome (1.0%), AIDS (0.5%), and ovarian cyst (0.5%) etc.

Clinical symptoms recorded from the cohort included dyspnea (93.9%), fever (65.2), gastrointestinal malaise (48.7%), cough (41%), neurological issues (34%) and urinary problems (12.2%). We also examined and documented the causes of death as per WHO guidelines [17], for both the sub groups of our cohort- one who died primarily...
due to COVID-19 and the other who died due to other likely diagnoses. The details have been tabulated in Table 2. Analysis of symptoms reported (for instance fever, cough, dyspnea, gastrointestinal malaise) and associated comorbidities (hypertension, diabetes mellitus, chronic kidney disease, chronic lung disease) showed significant association with patients who “died due to COVID-19” (p value < 0.0001). Patients that “died due to other likely diagnosis” were associated with decreased urine output, jaundice, pancytopenia etc. and congenital heart disease, autoimmune hepatitis, nephrotic syndrome etc. (p value = 0.0011).

**Discussion**

The safety of the personnel exposed to SARS-CoV-2-contaminated human remains is in apparent jeopardy [19]. As a first systematic evaluation of COVID-19 infection among mortuary and cemetery workers in Qatar, Alishaq et al. reported high SARS CoV-2 infection/ exposure based on PCR and serological testing [20], where the authors mention that the role of environmental contamination in transmission is difficult to establish. While there are no well documented cases of occupational exposure, transmission of viral infection cannot be ruled out during aerosol generating procedures (AGPs) like autopsies. Movement and manipulation of bodies during transfer and/or preparation causes shifting of the respiratory cavity leading to unanticipated emission of bodily fluids. Workers performing autopsies and/or collecting samples have been designated as the highest risk category among death care occupations by the U.S. Occupational Safety and Health Administration’s (OSHA), for they are routinely exposed to contaminated surfaces, respiratory droplets or body fluids of cadavers [21]. Many of the relatively few autopsies that have been performed worldwide have been carried out in BSL-3 autopsy suites [21,22], but economic feasibility of BSL-3 autopsy suites poses a barrier in third-world countries.

This study provides an overview of 199 COVID-19 positive decedents who were successively tested using nasopharyngeal swabs for the SARS-CoV-2 RNA after death, over a period of 0–120 hours. While all patients, both in-hospital and brought-dead, tested RT PCR positive for COVID-19 immediately after death, only 115 of the total subjects (58%) were found to have tested RT PCR positive when examined at different time points. COVID-19 is primarily an upper respiratory tract virus and targets the respiratory and vascular systems. As reported by Stokes et al. the predominant symptoms were fever, cough and dyspnea [23]. Moreover, as reported by Cascella et al. patients with comorbidities, for instance obesity, diabetes mellitus, cardiovascular disease, chronic kidney disease, chronic liver disease, develop severe COVID-19 and its associated complications. This is concordant with the findings in our cohort [24].

We observed that the SARS-CoV-2 viral RNA was still detectable in human remains after a substantial time which has been shown in previous studies [25]. However, our study demonstrated a gradual decrease in the viral load with increasing time after death (Fig. 2), which may be explained as follows: with cellular death in the post mortem setting, susceptible live cells are not available for the obligate intracellular organism to multiply and infect, leading to a cessation of viral replication. Thus, it is expected to show an overall decrease in the percentage of post-mortem nasopharyngeal swab samples testing PCR positive. However, there was a minor exception at T2 (24–48 h). Servadei et al., also reported an increase in the viral load in a significant number of patients 24 h after death and explained it using the hypothesis dubbed “twilight of death” [25]. The latter refers to a time window between death and decomposition, where absolute cellular death has not yet occurred. It is suggested that death is a slow fading away process rather than a simple switch-off, wherein alive and competent human cells are possibly infected by SARS CoV-2 and release viral particles in the absence of immune cells. Thus, pathological infections could continue, or even increase, in the first few hours after death [26].

The rate of percentage decrease in positivity (the slope of the percent positivity curve) for patients who died due to COVID is approximately 0.38%/hour. Through extrapolation of the plot on the x-axis and using the equation of a straight line (y = mx + b) in the graph, we calculated the number of days in which we get near 0% positivity, arriving at a value of 219 h, that is 9 days. As for those who died due to other diagnoses, the rate of fall of positivity is 0.56%/hour, reaching a plausible 0% positivity at around 166 h i.e., about 7 days after death. As reported by van Kampen et al., patients with critical COVID-19 may shed infectious virus for longer periods of time compared to what has been reported for in patients with mild COVID-19 [27].

The data on mean Ct value with time compliments this observation. Thus, the likelihood of a positive test result decreases with time of sample collection after death. Evidently, the SARS CoV-2 RNA lurks longer in those decedents who died due to COVID-19 and this information could contribute towards proper diagnosis of cause of death and lead to more accurate documentation for upcoming statistical studies.

When we evaluated the trend of decrease in RT-PCR positivity, the difference was non-significant for the “died due to COVID-19” sub-group. Patients that “died due to other likely diagnoses” showed a tendency towards statistical significance. This is likely because the decedents in “died due to other likely diagnosis” was evenly distributed among various time groups. In the “died due to COVID-19” sub-group, the number of decedents is not evenly distributed among the time groups and in the later time groups the number of decedents was less due to unavailability of cadavers. Nonetheless, trend line analysis showed a general decrease in RT-PCR positivity in both sub-groups.

It is important to note here that mere RNA detection does not equate to viability and infectious risk. Though a few studies have shown that low Ct values may be used as a surrogate for viability of virus and potential infectiousness, the possible infectivity is likely to be lost before the PCR test turns negative. Gabrielli et al. has earlier
reported the detection of SARS CoV-2 viral genes in the corpse of an exhumed infected person, one month after death. The drawn organs, lung and heart were positive by molecular test with Ct values of 31 and 36, respectively, and failed to grow in cell culture isolation [28], implying that the virus would be non-infectious at these Ct values.

Another interesting observation was the relation of Ct value with cause of death. Patients who had died primarily due to COVID-19 had higher viral loads (reflected from low Ct value) than those who died due to other likely diagnoses. This also tallies well with the observation that SARS CoV-2 RNA persists for a greater duration in corpses who had died primarily due to COVID-19 than otherwise (Fig. 3).

For potentially fatal respiratory secretion-transmission viruses, safety protocols should focus on minimizing exposure to bodily fluids from the decedents of severe febrile illness [8]. Various governmental bodies, including The Ministry of Health & Family Welfare (Govt. of India), the Italian Ministry of Health and the German Federal Ministry of Health, issued guidelines that discourage autopsies on COVID-19 positive bodies [15,29]. Nonetheless, some autopsies are inevitable for medico-legal and scientific purposes. Previous studies have shown that at an ambient temperature of 30 °C or higher, the risk of infection decreases, whereas refrigeration promotes survival of the virus [30,31]. Nevertheless, bodies are not generally kept at 30 °C for a long time, but are instead refrigerated to prevent decomposition. Hence, autopsy suites are to be designed with airflow control and airborne infection control provisions and personal protective equipment are required.

Limitations

Firstly, owing to ethical reasons we could not keep the same bodies for the entire duration of the study and sequential testing from the same source was not possible. This had two effects: i) number of bodies available at different time periods was unevenly distributed; ii) number of bodies at longer durations post death was fewer. Both these factors led to an inability towards establishing statistical significance in the “died due to COVID-19” group [32]. Secondly, viral culture studies and expression levels of viral proteins are required to evaluate virus survival. Mere presence of viral RNA is not always a true indicator of live virus, although some recent studies have also revealed that RT-PCR Ct values may correlate well with live virus till a certain extent [33].

Conclusion

Several questions arise pertaining to civil contingency plans with regards to management of COVID-19 bodies. The U.S. Occupational Safety, Health and Working Conditions Code, 2019, states that every employer shall ensure that the workplace is free from hazards which cause or are likely to cause injury or occupational disease to the employees. In a step toward this goal, our study was carried out with the aim of mitigating risks associated with healthcare professionals and utility of diagnosis in forensic investigation. We concluded that the percentage PCR positivity decreases with time. Therefore, after a unanimously prescribed time gap, it might be relatively safe to handle confirmed COVID-19 decedents, nonetheless following appropriate COVID-19 etiquettes throughout.

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Ethics approval and consent to participate

The study was approved by the Institute Ethics Committee (IEC), AIIMS, New Delhi (Ref. No.: RP-22/2020).

CRediT authorship contribution statement

Meenakshi Sharma, Megha Brijwal, Nabarun Chakraborty helped in Conceptualization, design, literature review, gaining ethical approval, data acquisition, manuscript layout, analysis, Writing - review & editing. Sharad Srivastav, Nirupam Madaan, Parin Lalwani helped in data acquisition and statistical analysis. Aashish Choudhary, Richa Agrawal, Arbind Kumar, Rajesh Malhotra helped in concept, design and review. Sanjeev Lalwani, Purva Mathur, Lalit Dar, Kapil Dev Soni, Anjan Trikha helped in concept, screening of intellectual content and review. The manuscript has been reviewed and approved by all authors.

Data availability

The datasets used and/or analyzed during the current study can be made available by the corresponding author on request.

Conflict of interest

All the authors report no conflict.

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